CHEMISTRY OF URINE

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A PRACTICAL GUIDE TO THE

ANALYTICAL EXAMINATION OF DIABETIC, ALBUMINOUS, AND GOUTY URINE

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PREFACE.

A CONSIDERABLE portion of the contents of this book was designed to foim part of the concluding volume of my "COMMERCIAL ORGANIC ANALYSIS" Vol III , Part 3id, of that work deals mainly with substances of animal oiigin, and will complete the chief liteiary work of my life But, of late, cii cumstances have led me to devote much attention to the chemical examination of urine, especially in ielation to certain pathological conditions of great importance in Life Assurance reports, as well as in clinical diagnosis and prognosis These examinations have caused me to investigate critically a large number of the analytical methods which are in vogue for the examination of urine, especially for sugai and albumin, and to confirm or disprove certain statements generally accepted as facts

The results of this extensive laboratoiy woik may be of assistance to many interested in Urinary Analysis Physicians who are called on to advise as to the acceptance or rejection of candidates for Life Assurance often find this duty very onerous Prognosis with regaid to patients who may be suffering from glycosuria or albuminuria is a difficult and

anxious task, and the more so as this task is one
upon the performance of which the patient's immediate
future is to cast so critical a light   Probably, also,
there are analysts who will welcome this collection of
analytical facts and methods

While attempting to bring the majority of the tests
and processes within the scope of everyday clinical
diagnosis, or of the reports required for Life Assurance,
I have also described other methods which cannot be
applied except by those accustomed to analytical
work, and who are possessed of the appliances of a
well-appointed laboratory

While desiring to give special prominence to the
methods of examining diabetic, albuminous, and gouty
urine, it appeared undesirable to omit all reference to
subjects of collateral interest, such as the proportions
of urea and total nitrogen in urine, the recent researches
on creatinine and on xanthine derivatives, and the
behaviour of urinary colouring matters   On the other
hand, the book is not planned as a *complete* guide to
Urinary Analysis   Thus, I have omitted all mention
of the methods of determining phosphates and most
of the other mineral constituents of urine, firstly,
because they are not of great pathological interest, and
secondly, because I have nothing to say about them
which cannot be found in every physician's and
analyst's library   Should this production meet with
such a reception as to call for the issue of a second
edition, it may be desirable to supply this and other
omissions

It is with great pleasure that I acknowledge the
valuable assistance rendered me by Dr James Edmunds,

of Dover Street, Piccadilly, to whom I am greatly
indebted for the perusal of the whole of the proofs,
and for many valuable suggestions, both scientific and
literary   I am also obliged to Messrs A W Gerrard,
F G Hopkins, and G Stillingfleet Johnson for the
perusal and correction of particular proof-sheets
Much time and labour have been devoted in my
laboratory to the examination of many of the tests
and processes described, and my cordial thanks are
due to Mr G Bernard Brook and Mr Arnold R
Tankard for their zealous and painstaking assistance
in this arduous work

The progress of further investigations which I have
in hand would be materially facilitated by increased
opportunities of examining abnormal specimens of
urine   I therefore take this opportunity of soliciting
the co-operation of clinical workers, who would greatly
oblige me by forwarding for examination specimens
of any urines which appear to justify such a course
There is reason to believe that some of the less known
constituents of urine, such as glycuronic acid and its
compounds, creatinine, xanthine, the indoxyl and skat-
oxyl derivatives, and particular pigments, are greatly
augmented under certain pathological conditions at
present not fully understood, and the systematic
examination of urines of abnormal character would
probably materially extend our knowledge of this
difficult and obscure subject   In sending such samples,
I would request that the urine be poured into a clean,
strong eight-ounce bottle, which should then be at
once securely corked, carefully packed, and distinctly
labelled, with the date and hour of passing, and with

the name and address of the sender. The sample should be forwarded at once by parcel-post, and full information as to the patient's history should be sent to me at the same time by letter-post   Of course the complete examination of urine for abnormal constituents cannot be effected on so small a quantity as eight ounces, but this amount will suffice to ascertain whether a more extended analysis of urine from the same source is desirable.

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GENERAL COMPOSITION OF URINE.

URINE is an excretion of extremely complex composition, even in a state of health, while pathological urine contains a variety of other bodies absent from or existent only in traces in the normal excretion

W D Halliburton gives the following list of constituents, the arrangement being based on Hoppe-Seyler's classification —

1 *Urea and related substances* Urea, uric acid, allantoin, oxalic acid, xanthine, guanine, creatinine, thiocyanic (sulphocyanic) acid

2 *Fatty and other non - nitrogenous substances* Fatty acids of the series $C_nH_{2n}O_2$, oxalic, lactic, and glycero-phosphoric acids , minute quantities of certain carbohydrates

3 *Aromatic substances* The ethereal sulphates of phenol, cresol, pyrocatechin (catechol), indoxyl, and skatoxyl , hippuric acid , aromatic oxy-acids

4 *Other organic substances* Pigments, ferments (especially pepsin), mucus, humous substances , and in dogs, cynurenic and urocanic acids

5 *Inorganic compounds* Sodium and potassium chlorides , potassium sulphate , sodium, calcium and

magnesium phosphates, silicic acid, calcium carbonate, ammonia salts

6 *Gases*   Nitrogen and carbonic acid.

In addition to the above constituents of normal urine, there may be present in certain pathological conditions —albumin and other proteids, hæmoglobin, methæmoglobin, bile-pigments, bile-acids, abnormal urinary pigments, leucine and tyrosine, oxymandelic acid, dextrose, milk sugar, glycuronic acid, fats, lecithin, cholesterin, cystin, constituents derived from food or drugs, and organised bodies, such as blood-corpuscles, urinary casts, and renal epithelium

Several other urinary constituents of considerable importance are omitted from Halliburton's list. Among these may be named the acetone, aceto-acetic ether, and hydroxy-butyric acid often present in diabetic urine during the latter stages of the disease; hypoxanthine, and substances resulting from the imperfect metabolism of food or tissue

The specific gravity of urine is roughly a measure of the contained solids

The constituents of urine of chief importance are the urea, uric acid, and phosphates, and it is to the determination of these that the quantitative examination of healthy urine is generally limited

Constituents of secondary importance are the creatinine, hippuric acid, chlorides, and sulphuric acid in the two forms of metallic sulphates and ethereal salts

In healthy urine, sugar and albumin exist only in traces, if at all, but in typical diabetic urine a variety of sugar is the leading constituent   Albumin sometimes co-exists with sugar in diabetic urine, but its presence is more characteristic of certain other diseases

The urine passed in twenty-four hours by a man

weighing 66 kilogrammes (= 144½ lbs) is stated by Parkes to measure 1500 c c (52 oz), and to contain about 72 5 grammes (or 2½ oz) of solids, having the following composition —

	Percentage Composition of Solids	Absolute Weight of Solids in Grammes	Weight per 1000 of Body Weight
Urea, CH_4N_2O,	45 75	33 18	0 5000
Creatinine, $C_4H_7N_3O$,	1 25	0 91	0 0140
Uric acid, $C_5H_4N_4O_3$,	0 75	0 55	0 0084
Hippuric acid, $C_9H_9NO_3$,	0 55	0 40	0 0060
Pigment and other organic substances,	13 79	10 00	0 1510
Sulphuric acid, SO_3,	2 77	2 01	0 0305
Phosphoric acid, P_2O_5,	4 36	3 16	0 0480
Calcium,	0 35	0 26	0 0004
Magnesium,	0 28	0 21	0 0003
Potassium,	3 45	2 50	0 0420
Sodium,	15 29	11 09	0 1661
Chlorine,*	10 35	7 50	0 1260
Ammonia,	1 06	0 77	0 0130
	100 00	72 54	1 1057
* = Sodium Chloride,	17 04	12 36	0 1852

According to J L W Thudichum (*Pathology of the Urine*, 1887), the average volume of urine excreted in twenty-four hours, by men weighing from 60 to 65 kilogrammes, is from 1400 to 1600 c c (48 to 56 fluid ounces), of a specific gravity averaging 1 020   The solids contained in this quantity of urine range from 55 to 66 grammes, and are stated to be composed as follow —

Urea,	30 to 40 grammes
Creatine,	0 30 }0 75 „
Creatinine, .	0 45 }
Xanthine and its derivatives,	undetermined
Uric acid,	0 50 „
Hippuric acid,	0 50 „
Cryptophanic acid,	0 65 „

Colouring matters,	. undetermined	
Biliary acids,	0 012	grammes
Oxalic, oxaluric, and nitrogenised deri-		
vative of sarco-lactic acid,	undetermined	
Acetic acid,	0 288	,,
Formic acid,	0 05	,,
Carbonic acid,	undetermined	
Sulphuric acid,	1 5 to 2 5	,,
Sulphur in other forms,	0 20	,,
Phosphoric acid as alkaline phosphates,	3 66	,,
Earthy phosphates,	1 28	,,
Lime,	0 17	,,
Magnesia,	0 19	,,
Iron,	undetermined	
Potassium and sodium chlorides,+	10 to 13	,,
Potash and soda,	undetermined	
Ammonia,	0 7	,,
Methylamine,	trace	
* Containing chlorine,	6 to 8	,,

Y v o n a n d B e r l i o z have published the results of numerous analyses of normal urine (*Lancet*, ii, 1888, page 629) Their mean figures are as follow —

	MALE		FEMALE	
	Per litre	Per diem.	Per litre	Per diem
Specific gravity,	1 0225		1 0215	
Volume,		1360 c c		1100 c c
Urea,	21 5 grms	26 5 grms	19 0 grms	20 5 grms
Uric acid, .	0 5 ,,	0 6 ,,	0 55 ,,	0 57 ,,
Phosphoric acid,	2 5 ,,	3 2 ,,	2 4 ,,	2 6 ,,

With the exception of the uric acid, which is almost the same for the two sexes, the amounts under each head are higher for males than for females The proportion of urea to uric acid is 40 1, and that of urea to phosphoric acid 8 1

W C a m e r e r (*Zeit Biol*, xxiv 306) has recorded the amount of total nitrogen contained in normal urine, and has compared it with that eliminated in the form of urea Thus the mixed urine from a

number of persons' measured on the average 1840 c c , had a specific gravity of 1 016, and contained —

	Pei diem.	Per cent
Total nitrogen, . .	16 06 grammes,	0 873
Nitrogen as urea,	14 15 ,,	0 769
Nitrogen in other forms, .	1 91 ,,	0 114

The nitrogen passed in the form of urea is about 90 per cent of the total

The foregoing analyses probably fairly represent the average composition of urine, but they take no account of the variation in composition resulting from change of food   The two following analyses by G B u n g e furnish interesting information in this connection   They were carried out on the urine of a young man in good health, who was fed in succession on animal and vegetable diet   The urine was collected on the second day, after an exclusive diet of roast beef, with a little salt and spring water   In the second case the urine was also collected on the second day, after an exclusive diet of wheat-bread, butter, a little salt, and spring water   Bunge points out that these analyses are probably unique among those published, in that all the important constituents were determined on the same sample of urine   The following were his results —

	Meat Diet	Bread Diet
Total measure of urine in 24 hours,	1672 c c	1920 c c
Urea,	67 2 grammes	20 6 grammes
Creatinine,	2 163 ,,	0 961 ,,
Uric acid,	1 398 ,,	0 253 ,,
Sulphuric acid,	4 674 ,,	1 265 ,,
Phosphoric acid,	3 437 ,,	1 658 ,,
Lime,	0 328 ,,	0 339 ,,
Magnesia,	0 294 ,	0 139 ,,
Potash,	3 308 ,,	1 314 ,
Soda,	3 991 ,,	3 923 ,,
Chlorine,	3 817 ,,	4 996 ,,

The figures for sulphuric acid were obtained by boiling the urine with hydrochloric acid and barium chloride, and hence include both that existing as metallic sulphates and that present as ethereal salts.[1]

It will be observed that the urea, creatinine, and uric acid are all greatly increased by a meat diet The phosphoric and sulphuric acids, which are largely derived from the oxidation of albuminoids, also show a marked increase.

[1] In the foregoing analyses the sulphuric acid and chlorine are sufficient to convert all the inorganic bases into sulphates and chlorides The ammonia was not determined, but if present in normal proportion ($i\,e$, from 0 4 to 0 9 gramme), it would suffice to convert the phosphoric acid into AmH_2PO_4 Further, the saturating power of the sulphuric acid is over-estimated, since some of it existed in the form of phenyl sulphuric acid, $C_6H_5\,HSO_4$, or allied compounds, exerting only a mono-basic function The quantity of sulphur in the form of ethereal salts in human urine averages one tenth of that present as metallic sulphates On acidulating the urine with acetic acid, and adding barium chloride, the metallic sulphates are precipitated If the liquid be filtered, and the filtrate rendered strongly acid by hydrochloric acid and boiled, the conjugated sulphuric acid salts are broken up, and the resultant sulphuric acid can then be thrown down as barium sulphate If the liquid be again filtered, evaporated to dryness, and the residue fused with nitre, an additional quantity of sulphuric acid is formed, corresponding, in human urine, to 10–20 per cent of the total sulphur excreted, but rising in anomalous cases to a larger proportion This unoxidised sulphur exists in the urine partly in the form of cystin, a body having the composition $C_3H_7NSO_2$, but a portion is excreted in the form of thiocyanates The ethereal sulphates are represented by the potassium salts of phenyl sulphuric acid, $C_6H_5\,HSO_4$, indoxyl-sulphuric acid, $C_8H_6N\,HSO_4$ (the so called urinary indican), skatoxyl-sulphuric acid, $C_8H_5(CH_3)N\,HSO_4$, and similar derivatives of catechol (pyro catechin) and quinol (hydroquinone)

PRELIMINARY EXAMINATION OF URINE.

VOLUME —The quantity of urine passed by a healthy man in twenty-four hours is commonly stated at about 50 oz (1450 c c), but the observations on which this statement is founded have been chiefly made on the inhabitants of gaols, workhouses, or barracks, where the inclination and opportunity for drinking is limited   Many men in a state of perfect health habitually pass a considerably larger volume than 50 oz daily   An excessive excretion indicates polyuria, and is a very common, but not invariable, accompaniment of glycosuria

Whenever practicable, all observations on urine should be made on portions of the mixed excretion of twenty-four hours, otherwise very misleading conclusions may be formed   Thus the acidity, specific gravity, colour, and other characters of urine vary greatly with the period of the day and the time which has elapsed since the last meal   In cases of diabetes of a mild type, or which are progressing towards recovery, sugar is often nearly or entirely absent from the morning urine, but returns for several hours after partaking of a meal of which carbohydrates have formed a portion

APPEARANCE —The *colour* of normal urine is pale yellow or amber   Typical diabetic urine is very pale, but this peculiarity appears to be due to its greater volume and consequent dilution of the urinary

pigments, as compared with the normal excretion, rather than to actual diminution of colouring matter

Dark urines owe their colour to bile-pigments, blood, tinctorial drugs (e g , rhubarb), or to excess of the pigments present in normal urine   The subject is discussed more fully in a subsequent section

URINARY SEDIMENTS — Normal human urine is clear, or contains a fine flocculent precipitate of mucus The urine of herbivorous animals is turbid when excreted, from the separation of earthy phosphates, and human urine is not unfrequently passed in the same condition   In certain diseases very turbid urine is voided, and may contain mucus, pus, tube-casts, epithelium, chyle, blood, &c

However clear the urine may appear when freshly passed, a deposit almost always becomes visible on allowing it to stand for a few hours.   In normal urine this deposit is nearly transparent, very light and flocculent, and consists of *mucus*   A heavy pulverulent deposit, of a buff or fawn colour, generally consists of *urates*, but *earthy phosphates* are not unfrequently deposited from urine of an alkaline reaction, A deposit of urates disappears on heating, while one consisting of phosphates is permanent, and often becomes denser on boiling the urine.

On standing for several days, normal urine deposits the whole, or at any rate the greater part, of its uric acid in the form of acid urate of sodium   On still further standing, the urine becomes alkaline from the decomposition of the urea with formation of ammonium carbonate.

ODOUR —The smell of urine is peculiar and slightly aromatic   The odour of the normal excretion is said to be due to phenol, and taurylic and damoluric acids The odour of diabetic urine often recalls that of hay, but in the later stages of the disease the smell of acetone is frequently perceptible   When cystin is

present the smell of the urine is at first like that of sweet-briar, but afterwards becomes offensive   Turpentine produces an odour like that of violets, and the essential oils of cubebs, copaiba, santal-wood, &c , when taken internally, communicate characteristic smells to the urine.   On long keeping, or when it has undergone fermentation in the bladder, urine has an ammoniacal odour, while an excretion containing blood or pus is often putrid, and occasionally evolves sulphuretted hydrogen

TASTE —The taste of urine is said to be at once salt and bitter   Diabetic urine has a distinctly sweet taste, owing to the presence of sugar, the existence of which was first recognised by this character   The French chemists still lay stress on the "saveur" as a test for urine

REACTION OF URINE —Normal urine has commonly a marked acid reaction to litmus, but during the so-called "alkaline tide" which follows meals the urine has not unfrequently a distinct alkaline reaction Diabetic urine is very generally acid, and the reaction is not readily altered by food or alkaline medicines

The acid reaction of urine has been attributed to the presence of traces of hippuric or other acids in an uncombined state, but is in all probability due to the occurrence of sodium dihydrogen phosphate (acid phosphate of sodium), NaH_2PO_4, produced by the reaction of the uric and sulphuric acids resulting from the metabolysis of the food on the basic sodium phosphate of the blood   Hence it follows that the acidity of urine is increased by muscular exercise and the consumption of highly albuminous food, as of course by the internal administration of mineral acids.

Normal urine becomes alkaline after a vegetable diet containing potassium salts of organic acids   Thus the acid potassium salts of tartaric, citric, malic, and

other vegetable acids are largely present in acid fruits, and on combustion are converted into potassium carbonate   Hence the urine passed after a fruit diet has a strong alkaline reaction and effervesces on addition of a mineral acid   Potatoes cause the urine to be strongly alkaline, because they contain but a small proportion of proteids, and hence yield but little sulphuric acid on oxidation, while on the other hand they are rich in potassium malate, which is converted into carbonate on ignition   The cereals and leguminous seeds, on the contrary, yield a urine as acid as that excreted under a meat diet, since they are rich in both albuminoids and phosphates [1]

The *alkalinity* of urine may be due to the presence of carbonates or basic phosphates of the alkali-metals, or to the presence of carbonate of ammonium   This last body is derived from the hydrolysis of the urea, according to the following equation — $CH_4N_2O + 2H_2O = (NH_4)_2CO_3$ This change occurs spontaneously in urine when kept for some days, and is owing to the action of a ferment called *torula ureæ*   If any such ferment find access to the bladder, as by the use of a septic catheter, decomposition may occur before the urine is voided, and the excretion may have an alkaline reaction to litmus even when freshly passed   The nature of this alkalinity may be readily ascertained by moistening red litmus paper with the urine   If the alkalinity be due to ammonia, the blue colour will disappear as the paper dries, but otherwise will be permanent

The quantitative determination of the *acidity* of urine may be made by titrating 100 c c of the sample

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[1] Reasoning from these facts, F Bunge regards cheese as a particularly unsuitable food for persons inclined to gravel, since in its manufacture the alkaline salts are largely lost in the whey, while the casein yields much sulphuric and phosphoric acid on combustion   Salted meat and fish he considers objectionable for similar reasons

with decinormal caustic soda, using phenolphthalein or alizarin as an indicator   The addition of the alkali is continued till a deep red colour is obtained with the former indicator or a violet with the latter   Each 1 c c of standard alkali required represents 0 012 gramme of sodium dihydrogen phosphate, NaH_2PO_4, to which salt the acidity of normal urine is very probably due

Similarly, according to F r e u n d and T o e p f e r,[1] the *alkalinity* of urine can be determined by titrating 100 c c with decinormal sulphuric or hydrochloric acid, the end-reaction being the point at which the red colour due to phenolphthalein disappears, or the violet of alizarin changes to yellow   Each 1 c c. of decinormal acid required is stated to represent, when the former indicator is used, 0 0106 gramme of sodium carbonate, Na_2CO_3, or 0 0164 of trisodium phosphate, Na_3PO_4 When alizarin is the indicator used, each 1 c c of acid represents half these quantities

Whatever variations in the reaction of urine may be observed hourly under the influence of food, &c , the mixed excretion of twenty-four hours, if of normal character, will invariably be found to exhibit an acid reaction

SPECIFIC GRAVITY AND TOTAL SOLIDS OF URINE — The specific gravity of normal human urine varies from 1015 to 1025, averaging about 1020 , but after great physical exertion and consequent profuse perspiration it has been known to be as high as 1035 in the case of healthy persons   On the other hand, after excessive drinking it has fallen as low as 1002   Typical diabetic urine is of very high specific gravity, usually exceeding 1030 and occasionally rising as high as 1074

The specific gravity of urine is most conveniently ascertained by means of the hydrometer, an instrument which, if accurately constructed, gives, with reasonable care, indications quite delicate enough for

[1] *Zeit. physiol Chem* , xix 84, and *Jour Chem Soc* , lxvi part ii page 260

ordinary purposes   It must be remembered, however, that the readings are very materially affected by alterations of temperature   Hence the urine should be brought as nearly as possible to a temperature of 60° F. ($=15 \cdot 5°$ C.), and on no account should the hydrometer reading be taken when the urine is sensibly warm [1]

The specific gravity of urine is roughly an indication of the proportion of solid matter contained in it, and hence of the total waste of the system.   It would be an accurate measure of this if the solids were of a homogeneous nature, but as solutions of equal strength of sodium chloride, urea, sugar, and other urinary constituents have different specific gravities, the density of the urine will vary with the *nature* of the contained solids, as well as with their *amount*

The following are the specific gravities at 60 F ($=15 \cdot 5$ C) of solutions of urinary constituents containing 10 per cent of the solids —

Substance	Specific Gravity	Observer
Sodium Chloride (common salt),	1073 35	Gerlach
Urea,	1028 2	A H Allen.
Dextrose,	1040 0	F Salomon.
Albumin,	1026 0	————

Hence to deduce accurately the percentage of solids from the gravity of urine it would be necessary to ascertain the proportion of each of the leading constituents ; but when that was effected, the specific gravity would no longer have any interest

If the specific gravity of a sample of urine above that of water ($=1000$) be multiplied by 2 33, accord-

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[1] F W. Fletcher (North London Chemical Works, Holloway) has devised a "thermo hydrometer" which allows the temperature of the liquid in which it is immersed being read with great facility   One side of the ivory scale enclosed in the stem indicates the specific gravity of the liquid, while the other shows the temperature ; the stem of a delicate thermometer, the bulb of which is situated below that of the hydrometer, running up inside the hollow hydrometer stem   An approximate correction for temperature may be made by adding one degree to the observed specific gravity for each 8° F above 60°.

ing to Haeser and Christison ; by 2, according to Trapp , or by 2 2, according to Loebisch , the products will be the number of grammes or grains of solids contained in 1000 c c or fluid grains of the sample By dividing the product by 1000, and multiplying by the volume in centimetres or fluid grains passed in twenty-four hours, the total weight of solids excreted by the kidneys in that time will be found [1] The variation in the factors given by the authorities quoted shows that, for reasons above stated, the method is only roughly approximate

Bouchardat has proposed a somewhat similar formula for ascertaining approximately the proportion of sugar contained in diabetic urine from the specific gravity of the liquid The gravity above 1000 is multiplied by 2, and the product by the number of litres of urine passed in twenty-four hours From the product 60 is deducted, this correction representing the influence of the solids other than sugar, when the remainder will represent the weight of sugar in grammes passed in twenty-four hours [2]

When an accurate determination of the solid matters of urine is required, it is best effected by evaporating an exactly weighed or measured quantity in a flat-bottomed porcelain or platinum capsule on the water-bath, exposing the apparently dry residue for some hours in the water-oven, and weighing the residue with precautions to prevent re-absorption of water 5 c c , or 100 fluid grains, is a suitable volume of urine to use for the determination

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[1] Thus if the urine passed in twenty four hours measure 1450 c c , and have a specific gravity of 1032 5, the total solid matters excreted by the kidneys in that time will be, by Haeser and Christison's formula,

$$\frac{32\ 5 \times 2\ 33 \times 1450}{1000} = 101\ 1 \text{ grammes}$$

[2] Thus, taking the same urine as before —32 5 × 2 = 65 0 , 65 × 1 45 = 94 25 94 25 − 60 = 34 25, which figure represents the weight in grammes of sugar excreted by the kidneys in twenty four hours

DIABETIC URINE.

Glycosuria, or the excretion of urine containing a kind of sugar known as glucose, is especially characteristic of the disease known as *Diabetes Mellitus* [1] It is commonly, but by no means invari-

[1] " According to the writings of Celsus, Aretæus and Galen, the disease termed 'diabetes' (διά, 'through,' βαίνω, 'I go') seems to have been recognised in a general way by the ancients The progressive emaciation characteristic of the malady was observed as being accompanied by inordinate thirst, voracious appetite, and excessive discharge of urine It was not, however, until 1674 that the urine in certain cases was discovered to possess a sweet taste, and the honour of this discovery, on which followed the establishing of the distinction between *diabetes insipidus* and *glycosuria diabetes*, is due to Willis, an English physician A hundred years subsequently, Dobson, of Liverpool, discovered that the blood as well as the urine contained sugar, and he inferred therefrom that this sugar was separated from the blood, and not formed by the kidney In 1778, Cowley separated the sugar from the urine in a free state In 1815, Chevreul pointed out that the sugar existing in the urine in cases of *diabetes mellitus* was different from cane sugar and closely resembled that of the grape, and in 1825 Tiedmann and Gmelin ascertained that during its passage along the alimentary canal starchy matter was transformed into sugar "—*Urine and Urinary Analysis*, by D Campbell Black

Glycosuria may be temporarily induced (so long as the liver is charged with glycogen) by puncturing the floor of the fourth ventricle of the brain, while the lesion of a closely adjacent part of the same ventricle has been found to produce polyuria Hence it has been suggested that in cases of diabetes in which both glycosuria and polyuria are present both these localities of the fourth ventricle have suffered irritation or injury

Complete removal of the pancreas from an animal produces a condition of diabetes, which is relieved if the pancreas from another animal be grafted into the abdomen of that from which the pancreas was extirpated Similarly, disease of the pancreas in man causes diabetes

Diabetes kills the person suffering from it by starving the tissues and emaciating the body Sometimes death ensues from uremia due to exhaustion of the kidneys and their consequent failure to excrete urinary materials from the blood

The physiology of diabetes is very imperfectly understood The appearance of sugar in the urine depends upon some failure at points where the sugar is

ably, associated with *Polyuria,* or the passing of an excessive volume of urine This may exist without the presence of glycosuria, in which case the disease is known as *Diabetes Insipidus* [1] In typical cases of *diabetes mellitus* the volume of urine excreted is frequently from 100 to 130 oz per diem, and sometimes reaches twice this volume An excretion of 400 oz per diem has been observed

Typical diabetic urine is very pale, probably owing to the dilution of the urinary pigments, but in mild cases, unaccompanied by marked polyuria, the urine has often a very dark colour The odour sometimes resembles that of hay, but in advanced cases of diabetes the excretion has often an odour of acetone or alcohol The taste of diabetic urine is distinctly sweet from the presence of sugar

The urine of diabetic persons has a tendency to froth on agitation Its reaction is usually distinctly acid, even after meals or alkaline medicines have been taken

The specific gravity of typical diabetic urine is very

absorbed and assimilated, or at some other point (*e g* , the muscular tissue) where sugar properly assimilated in the blood fails to be oxidised and made use of Sugar injected directly into the blood is not oxidised in the muscle, but runs off through a healthy kidney and is found in the urine The significance of sugar in the urine of those who eat redundantly of sugar and other carbohydrates, who take little muscular exertion, and whose persons are already laden with fat, is quite different from that which it has in the case of persons who are emaciated Similarly, sugar in the urine of an over-charged system is very different in its significance from glucose in the urine of the same person when all sugar has been stopped, the bulk of the starch has been excluded from the diet, and a fair amount of daily walking or other gentle muscular exercise is taken

[1] T h u d i c h u m mentions a case in which 5600 c c (=196 oz) of urine containing 0 8 per cent of sugar was excreted in twenty four hours, while at a later period the volume fell to 4333 c c , and contained only traces of sugar

In many cases of diabetes with a minimum excretion of sugar, no material increase in the volume of urine is observed, though the patients have a strong desire to micturate In these cases it is assumed that the sugar exerts an irritating action on the bladder, and causes its frequent evacuation In many cases polyuria appears to be the result of an irritation of the nerves and consequent congestion of the kidneys

high, the usual range being from 1030 to 1040; but a density of 1065 was observed by S e e g e n in a urine containing 10 per cent. of sugar.[1] B e r n a r d states the maximum density at 1074.

Albumin is sometimes present in the urine in chronic cases of diabetes, but tube-casts are of rare occurrence

Unless previously concentrated, diabetic urine does not usually deposit uric acid when acidified and left at rest   Whether this behaviour is due to the dilute condition of the excretion, or to the presence of less than the usual amount of uric acid, is not certain.

As a rule, diabetic persons excrete more urea than persons in health, owing to the greater amount of proteids they consume   There is no relation between the urea and the sugar excreted.   The latter varies with the amount of starchy and saccharine food taken, except in cases where sugar is produced under a strictly albuminous diet

Dieting experiments on diabetic patients show that more proteids are used than by healthy persons, since the carbohydrates are not available as a proteid-saving food   More fat undergoes combustion in the system of diabetics than in that of normal persons   Muscular work is stated to increase the excretion of urea, but not to affect sensibly the elimination of sugar

The quantity of sugar excreted by diabetic persons varies from mere traces to as much as 600 grammes (20 oz) in the twenty-four hours   The percentage of sugar present is, of course, dependent on the volume of the urine   In severe cases it not unfrequently reaches 8 or 10 per cent , and, according to A H H a s s a l l, as much as 15 per cent is present in some

[1] As a 10 per cent solution of pure sugar in water has a specific gravity of 1040, the difference between this figure and the observed density may be regarded as due to the normal constituents of urine   Thus, eliminating the effect of the sugar, the urine would have had a specific gravity of 1025

cases   The morning urine contains least sugar, while
that passed three or four hours after a meal is the richest
in sugar   Under strictly animal diet, the sugar will
often fall to 1 per cent, while a meal of starchy or
saccharine food will raise it to five or even to ten
times that amount   Hence the pathological signifi-
cance attaching to the excretion of 50 grammes of
sugar daily when under strictly animal diet is much
greater than the elimination of a larger amount when
saccharine and amylaceous foods are being freely
taken

As the proportion of sugar in diabetic urine varies
largely according to the time of day and the nature
of the food taken, it is highly important that any
analytical examination should be conducted on the
united excretion of the previous twenty-four hours

The question of the occurrence of traces of sugar in
normal human urine has been the occasion of much
controversy, and the last word on the subject still
remains to be said   B r u c k e (*Wien Akad Sitzung-
ber*, xxix 346) appears to have been the first to state
that all normal urine contained sugar, and this view was
supported by B e n c e J o n e s (*Jour Chem Soc*, xiv
22), K u h n e, T u c h e n, and many other observers ;
but opposed by F r i e d l a n d e r, W i e d e r h o l d,
M a l y, and K u l z   The question was re-examined
in 1871, by S e e g e n, who pointed out many fallacies
in the methods of those who had found sugar, and
concluded that it was either absent from normal
human urine, or present in such small proportion that
the then existing methods were insufficient for its
positive recognition in the presence of co-occurring
substances which simulate many of its reactions   On
the other hand, F W P a v y, in 1878, concluded that
sugar was a normal constituent of urine, and that no
sharp line of demarcation could be drawn between the

excretion in health and in diabetes, except quantitatively (*Guy's Hospital Reports*, xxi 413). M o l i s c h, from the examination of a large number of samples of healthy human urine by the alpha-naphthol and thymol tests, came to the conclusion that traces of sugar are met with frequently in human urine , but the value of his tests, and hence the accuracy of his conclusions, have been disputed by Leuken and also by S e e g e n (*Jour Soc Chem Industry*, vi 149, 150). E L u t h e r, again (*Chem Centr* , 1891, ii 90, and *Jour Chem Soc* , lx 1559), as the result of the application of the furfuraldehyde and alpha-naphthol tests to a large number of samples, concluded that glucose is present in all human urine, the amount found in the excretion of adults averaging 0 1 per cent , while the total carbohydrates amount to 0 2 per cent According to E R o o s (*Zeit physiol Chem* , xv 513), the normal urine of the dog, horse, and rabbit always contains more or less carbohydrates, as indicated by the furfuraldehyde reaction and confirmed by the benzoic chloride test Human urine is stated by Roos always to give an affirmative reaction with phenyl-hydrazine, and the same is true of dogs' urine, while the excretion from rabbits gives especially well-formed crystals The urine of all these animals was found to be slightly lævo-rotatory W e d e n s k i (*Zeit physiol. Chem* , xiii 122), by agitating a large volume of urine with benzoic chloride, obtained a separation of the insoluble benzoyl compounds of carbohydrates On separating the precipitate and treating it with soda, a portion dissolved and appeared to consist of animal gum, while the undissolved portion gave the reactions of dextrose

G S t i l l i n g f l e e t J o h n s o n denies the presence of traces of sugar in normal urine, on the ground that strictly negative reactions were obtained on testing

the filtrate from the precipitate produced on treating the urine with mercuric acetate   But while mercuric acetate effectually and conveniently removes from solution the creatinine and other interfering substances, it also exerts an oxidising action on glucose itself, so that where mere traces are present they might be expected to suffer complete destruction.   Direct experiments, made by the author to test the point, showed that the oxidation of the glucose was but trifling in the highly dilute solutions employed   The writer, by what was *apparently* substantially the same method as that by which G S Johnson obtained negative results, but confirmed by the results of the phenyl-hydrazine test,[1] satisfied himself that minute quantities of sugar are present in some specimens of urine from persons apparently in perfect health

Similarly, relying on the failure of normal urine to yield a red-brown coloration with an alkaline solution of picric acid, after the creatinine, &c , have been removed with mercuric acetate, S i r G J o h n s o n maintains strongly the absence of sugar from the excretion , and meets the objection that such urine often gives a crystalline product with phenyl-hydrazine by the suggestion that, "for some reason, the test must be difficult to apply and uncertain in its results "[1]   (See articles and correspondence in the *Lancet* during July and August, 1894)   It is contended that the mere presence of traces of sugar in urine thereby proves the excretion to be abnormal, though sugar admittedly appears temporarily on very slight provocation [2]   But such an argument begs the

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[1] Glycuronic acid and its compounds give with phenyl-hydrazine a crystalline compound closely resembling phenyl glucosazone, but having a different melting point, but with proper precautions no confusion between the two bodies is possible   (See pages 41 and 88)

[2] In the discussion of a paper by the author, "On the Examination of Urine for Small Quantities of Sugar," read before the Society of Public Analysts, Mr G Stillingfleet Johnson is reported to have said —" The question was,

very question at issue, which is whether normal urine does not sometimes contain traces of sugar.

The latest experimental contribution to the controversy is by F. W Pavy (*Physiology of the Carbohydrates*, 1894), who, by operating on large quantities of urine, precipitating the sugar in combination with oxide of lead, and recovering it from this compound, obtained a liquid which exhibited the chief chemical reactions of glucose   No attempt appears to have been made to obtain the glucose in a solid form, and the method employed for effecting its fermentation by yeast is open to criticism [1]

W D Halliburton (*Chemical Physiology and Pathology*, 1891) considers the balance of evidence clearly in favour of the existence of a small quantity of sugar in normal urine.[2]

What is a normal urine? This was simply reduced to the further question, Who is a healthy man?   Everybody knew that a man might rise in the morning a healthy man, and that he might go to bed at night anything but a healthy man   He was quite sure that slight errors of diet—such, for instance, as taking a late dinner, or dining out—were sufficient to produce a temporary glycosuria, which was of no importance whatever   If the test were applied carefully to the urine of healthy individuals, it would be found in the long run to give practically negative results   He had no doubt that the reason why the idea that normal human urines were saccharine in character had existed so long was that the reducing action of creatinine had been mistaken for the reducing action of sugar "—*Analyst*, xix 185

[1] See a correspondence in the *Lancet*, January, February, and March, 1895

[2] In a letter published in the *Lancet* for February 9th, 1895, Halliburton writes —" I think that a careful study of the researches of Seegen and of Baisch, in which more stringent chemical methods were employed than in those of Dr Pavy, will convince the impartial observer that a small quantity of glucose is obtainable   Whether this is artificially produced by the method of analysis, as suggested by Sir George Johnson, is a subject that demands renewed research   Dr Pavy gives the percentage of sugar in normal urine about five times greater than has been found by these observers, and I think his high figure is most easily explicable by Sir George Johnson's hypothesis— namely, a neglect to take into consideration the reducing action of creatinine "   This attempted explanation does not appear valid, since Pavy relies largely on the separation of the glucose in the form of an insoluble lead compound   It is nevertheless remarkable that not the slightest reference to creatinine or its proved reducing power on copper solutions, &c , is to be found throughout Dr Pavy's writings, and in a letter published in the *Lancet* for March 2nd, 1895, Pavy declines a direct challenge of Dr Halliburton to explain his views as to creatinine

THE CARBOHYDRATES OF URINE.

The "sugars" belong to the class of organic compounds known to chemists as C a r b o h y d r a t e s This name is a survival of the period when they were regarded as hydrates of carbon, or compounds of carbon with the elements of water, if not with water itself Thus the carbohydrates contain carbon, hydrogen, and oxygen, the two latter elements being in the proportion in which they exist in water Hence the carbohydrates, as a class, may be represented by the generic empirical formula —$C_xH_{2n}O_n$ Great progress has been made of late years in the knowledge of the constitution of sugars and other carbohydrates, the researches of E m i l F i s c h e r being of pre-eminent importance. Fischer has shown that the class of sugars known as glucoses, of which grape-sugar is the type, are really the a l d e h y d e s or k e t o n e s of the hexatomic alcohol mannite or mannitol, having the formula —$C_6H_8(OH)_6$ Just as aldehyde is obtained by oxidising ordinary a l c o h o l, C_2H_6O, so from m a n n i t o l, $C_6H_{14}O_6$, the corresponding compound, $C_6H_{12}O_6$, is obtained The carbohydrates of this composition are called *Monosacchards*, the best known members of the class being d e x t r o s e (sometimes called dextro-glucose, or simply glucose), l æ v u l o s e, and g a l a c t o s e By the loss of the elements of water, two molecules of these glucoses may coalesce to form *Disacchards*, thus ·—$2C_6H_{12}O_6$ −$H_2O = C_{12}H_{22}O_{11}$ The disaccharides thus formed, when boiled with dilute acids, readily take up the elements of water again, becoming split into two molecules of monosacchard These two molecules may be either identical or dissimilar in nature Thus, maltose splits into two molecules of dextrose cane-sugar yields one of dextrose and one of lævulose while milk-sugar is hydrolysed to dextrose and galac-

tose. *Trisaccharids*, resulting from the coalescence of three molecules of glucose, are known, the typical member of this class being r a f f i n o s e, $C_{18}H_{32}O_{16}$

S t a r c h and d e x t r i n are still more complex molecules, but, like the more simply constituted saccharides, tend to hydrolyse into their constituent disaccharide and monosaccharide molecules under the influence of dilute acids or other hydrolysing agents The following represents the chief features of existing knowledge on the subject —

Saccharid	Hydrolysis Products
α- and β-Amylan	Dextrose
Cellulose	Dextrose
Dextrin	Dextrose
Glycogen	Dextrose
Inulin	Lævulose
Lichenin.	Dextrose
Tunicin	Dextrose
Starch	Dextrose
Maltose	Dextrose
Lactose (milk sugar)	Dextrose and Galactose
Cane-Sugar	Dextrose and Lævulose
Raffinose	Dextrose, Lævulose, and Galactose

Dextrose   Dextro-glucose   Glucose   Grape-sugar $C_6H_{12}O_6$, $CH_2(OH)(CHOH)_4 COH$,   or, $CH_2(OH) CH(OH) CH(OH) CH(OH) CH(OH) COH$ [1] Dextrose is a constant, or at least a very frequent, product of the hydrolysis of the polysaccharides by boiling with dilute acids   It is also produced by the hydrolysis of the natural glucosides amygdalin, populin, salicin, hesperidin, lupulin, phloridzin, and ruberythric acid   Æsculin, arbutin, and coniferin also yield dextro-rotatory glucoses on hydrolysis, but it is not certain that they are identical with

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[1] This constitutional formula shows the presence of the COH group, and consequently classes dextrose among the a l d e h y d e s   On the other hand, lævulose contains a carboxyl group, CO, and has the constitution of a k e t o n e —$CH_2(OH) CH(OH) CH(OH) CH(OH) CO CH_2(OH)$

ordinary dextrose   On the other hand, lævulose is obtained when inulin is boiled with dilute acids Dextrose occurs in the free state in grapes, many other fruits, the seeds and sap of plants, &c   It also occurs in the blood, liver, and other parts of the body, and is the characteristic sugar of diabetic urine [1] Dextrose often crystallises from honey, in which it co-exists with lævulose   As already stated, a mixture of dextrose and lævulose in equal proportions results from the action of dilute acids on cane-sugar, and it is also formed by the hydrolysis of milk-sugar and maltose

Dextrose usually crystallises from its aqueous solution in granular hemispherical warty masses or tabular crystals, containing $C_6H_{12}O_6 + H_2O$, but hot concentrated solutions often deposit anhydrous dextrose in prisms   Dextrose loses its water of crystallisation when gently heated   The anhydrous substance melts at 146°, and at about 170° C loses water and is converted into d e x t r o s a n, $C_6H_{10}O_5$, and at higher temperatures (200° C) yields c a r a m e l

Dextrose is less soluble than cane-sugar in cold water, requiring $1\frac{1}{3}$ times its own weight, but it dissolves in all proportions in boiling water, forming a syrup having a sweetening power inferior to a solution of cane-sugar or lævulose of the same strength

Solutions of dextrose exert a powerful dextrorotatory action on polarised light, and one of the most accurate methods of estimating dextrose, in the absence of other optically active substances, is based on this fact   The specific rotation of dextrose solutions for a concentration of 10 per cent is $+52\ 7°$ for the sodium ray, and $+57\ 0°$ for the transition-tint

When treated with yeast and exposed to a moderate temperature, solutions of dextrose readily undergo the

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[1] It is not absolutely certain that the dextro rotatory glucose of diabetic urine is strictly identical with the sugar of grapes

alcoholic fermentation, the weight of alcohol generated being approximately half that of the glucose fermented. The best conditions for obtaining quantitative results are described on page 46, *et seq*

Dextrose is not affected when heated for a moderate time with dilute acids. Prolonged treatment is said to result in the formation of products having the probable formula $C_6H_{14}O_7$. When quite pure, dextrose is not readily charred by concentrated sulphuric acid, but combines with it to form a compound decomposed by water By treatment with nitric acid, dextrose is oxidised to s a c c h a r i c a c i d, $C_6H_{10}O_8$

When a caustic alkali is added to a solution of dextrose, the liquid acquires a colour ranging from yellow to dark brown, according to the amount of glucose present The change of colour occurs slowly in the cold, but almost instantaneously on heating The reaction results in the formation of acetal, acetone, and formic, acetic, and lactic acids, besides the little-known bodies termed glucinic, japonic, and saccharumic acids On acidulating the brownish liquid with nitric acid, the colour is lessened or destroyed, and the solution acquires the characteristic odour of caramel (burnt sugar)

M o o r e applied the foregoing facts to the detection of sugar in diabetic urine The sample to be tested is first freed from albumen, if present, by rendering it faintly acid with acetic acid and boiling The filtered liquid, or, in the absence of albumin, the original urine, is mixed with an equal measure of normal caustic soda or potash (or *Liquor potassæ*, B P), and filtered, without heating, from the precipitated earthy phosphates The filtered liquid is then boiled in a test-tube, in such a manner that only the upper part is heated, when in presence of much glucose a yellow or brown colour will be developed in the heated part of

the solution, and contrasts well with the unchanged lower portion   The test is simple, and occasionally very useful, but does not indicate with certainty less than 2 per cent of glucose   Cane-sugar, uric acid, and creatinine give no similar reaction   Urine containing the colouring matters of rhubarb and senna becomes reddish-brown with alkali before heating, while samples containing catechol (pyrocatechin) acquire a brown colour on exposure to air

A solution of dextrose dissolves the alkaline earths, forming yellow solutions precipitated by alcohol   By boiling with excess of lime dextrose is rapidly acted on and destroyed

Dextrose, when pure, is not precipitated by neutral or basic lead acetate, but on subsequent addition of ammonia it yields a white insoluble compound of dextrose and lead hydroxide, which may be washed without decomposition if carbonic acid be excluded This lead compound affords a means of isolating dextrose from urine and freeing it from various co-occurring substances   (See page 43)

When heated with water and silver oxide, dextrose yields glycollic, oxalic, and carbonic acids, but not acetic acid

Dextrose is a powerful reducing agent, especially in presence of free alkali and at a high temperature Thus at the boiling-point, in alkaline solution it reduces silver, mercury and bismuth to the metallic state, cupric oxide to cuprous, ferricyanides to ferrocyanides, blue indigo to white indigo, &c , &c   Further details of these and similar reactions, and their application to the detection of urinary sugar, are given on page 50, *et seq*

By the action of nascent hydrogen in neutral or alkaline solution, but not in presence of acid, dextrose is reduced to m a n n i t o l, $C_6H_{14}O_6$

With phenyl-hydrazine dextrose reacts to form a well defined o s a z o n e, crystallising in tufts of needles The properties and method of preparing this important body are fully described on page 86, *et seq*

On agitating a solution of dextrose with benzoyl chloride, a compound of the two separates in white crystals This reaction has been employed to demonstrate the presence of glucose in urine

LÆVULOSE LÆVO-GLUCOSE FRUIT SUGAR. $C_6H_{12}O_6$, or $CH_2(OH) CH(OH) CH(OH) \dot{C}H(OH) CO CH_2(OH)$. Lævulose occurs with dextrose in honey and in many fruits, and is a product of the hydrolysis of cane-sugar and raffinose The hydrolysis of the starch-like body i n u l i n yields lævulose without dextrose. The conversion of dextrose into lævulose has been effected by E Fischer by acting on the former with phenyl-hydrazine, reducing the resultant glucosazone to iso-glucosamine by zinc and acetic acid, and treating the last body with nitrous acid

Lævulose is separated from dextrose by converting them into the lime compounds, when the former sugar forms an insoluble product, which may be separated from the soluble lime dextrosate, washed, and decomposed by oxalic acid

Lævulose is generally described as a syrup, but on treating this with absolute alcohol the lævulose separates after some time as a mass of fine shining needles, which melt at 95° and undergo partial decomposition at 100° C

With phenyl-hydrazine in excess, lævulose reacts to form an osazone said to be identical with that yielded by dextrose under similar treatment

In its other chemical reactions lævulose presents the closest resemblance to dextrose, but differs from it in the characters of its lime compound as already mentioned, and in being readily affected by treatment

with dilute acids, or even by boiling its aqueous solution   Formic, propionic, and other acids are formed by boiling lævulose with dilute hydrochloric or sulphuric acid

Lævulose also differs from dextrose in the product of the action of bromine   When a solution of dextrose is heated with bromine-water, and the liquid then treated with silver oxide (care being taken to avoid an excess of the latter), g l u c o n i c   a c i d, $HC_6H_{11}O_7$, is formed, and may be obtained as a syrup on evaporation   If slaked lime be added in excess to its lukewarm solution, and the liquid filtered and heated to boiling, the acid is almost completely precipitated as a basic calcium gluconate   When lævulose is similarly treated with bromine-water and oxide of silver, it yields g l y c o l l i c   a c i d, $HC_2H_3O_3$, the calcium salt of which crystallises in silky needles, which require about 90 parts of cold water or 18 of boiling water for solution

The most characteristic distinction between dextrose and lævulose is their action on polarised light.   Thus, while dextrose, as its name denotes, exerts a dextrorotatory action, lævulose is still more strongly lævorotatory ; and while the optical activity of dextrose is unaffected by temperature the activity of lævulose rapidly diminishes as the temperature increases   Thus at about 87° C its specific rotation for the D ray is −52 7°, being exactly the same as that of dextrose, but in the opposite direction   At 20° C , H o n i g and J e s s e r give the specific rotation as −90 72° for a 10 per cent. solution   J u n g f l e i s c h and G r i m b e r t (*Compt rend* , cvii 390) give the following formula for the specific rotation of solutions of lævulose for temperatures (*t*) between 0° and 40° C and concentrations (*p*) less than 40 per cent —

$$[a]_D = -101\ 38 - 0\ 56t + 0\ 108\ (p-10)$$

The rotation decreases by 0 56 degrees for every rise of 1° C in temperature   In a later paper (*Compt rend*, cviii 144) they find that the rotation of lævulose prepared from mulin is − 90 02°, while that obtained from invert-sugar exhibits a rotation of − 95 74°, both at a temperature of 20° C   This they attribute to the influence of the mineral acid used for inverting the cane-sugar, the same difference not being observed when acetic acid was substituted, but becoming at once apparent on adding hydrochloric acid   O s t (*Berichte*, xxiv 1636) has to some extent confirmed the foregoing observations, and gives the following formula for the specific rotation of pure lævulose at 20° C p is the weight in grammes of lævulose in 100 grammes of the solution —

$$[a]_D^{20} = -(91 \ 90° + 0 \ 111p)$$

Lævulose is stated by some observers to occur at times in the urine of diabetic persons   Its presence has been commonly inferred from discordance between the estimations of glucose by the polarimetric and oxidation methods   But as lævulose does not appear ever to have been actually isolated from urine, the evidence of its presence is at best inconclusive   On *a priori* grounds, the existence of lævulose in urine under ordinary conditions is improbable, as it is much more oxidisable than dextrose   This fact is so well-established that the use of lævulose is actually permitted to diabetic patients[1]   According to J B H a y c r a f t, patients suffering from chronic diabetes can oxidise 50 grammes $\left(= 1\frac{3}{4} \text{ oz}\right)$ of lævulose per diem   In some acute cases, a portion of the lævulose is burnt off, a second converted into and

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[1] Lævulose is now an article of commerce, and can be obtained at a price of 3s 6d per lb from Messrs A & M Zimmermann, 6 and 7 Cross Lane, London, E C

excreted as dextrose, while a third passes into the urine unchanged The same result may be expected from the ingestion of cane-sugar In rabbits, lævulose undergoes conversion into glycogen, which accumulates in the liver

H Leo (*Chem Centr*, 1887, page 193) isolated from diabetic urine a reducing substance which, after being purified from the co-occurring dextrose by repeated treatment with a solution of barium hydroxide in methyl alcohol, was obtained as a bright yellow syrup It was lævo-rotatory ($a_D = -36\ 07°$), was not fermentable with yeast even after boiling with hydrochloric acid, and had a cupric oxide reducing power much less than that of dextrose The body was a carbohydrate of the formula $C_6H_{12}O_6$

MILK-SUGAR or LACTOSE, $C_{12}H_{22}O_{11},H_2O$, has been found in the urine of nursing women by Biot, Kaltenbach, Sinetz, and others It was isolated by Hofmeister (*Zeit physiol Chem*, 1 101) by precipitating the liquid with neutral lead acetate, treating the filtrate with ammonia, and precipitating the refiltered liquid once again with lead acetate and ammonia These two last precipitates were decomposed by sulphuretted hydrogen, the filtrate

Fig 1 —MILK SUGAR

shaken with oxide of silver, and the filtered liquid freed from silver by sulphuretted hydrogen Barium carbonate was next added, and the liquid evaporated On treating the residue with alcohol, the milk-sugar was dissolved, and obtained in a crystalline form by evaporating the solution *in vacuo* over sulphuric acid (See fig 1) Kaltenbach obtained mucic acid and galactose from the body thus isolated, thereby conclusively identifying it with milk-sugar
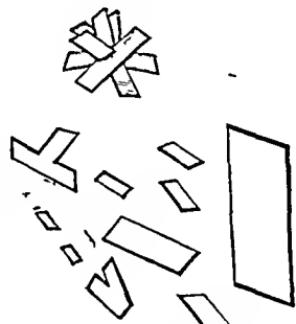
Milk-sugar yields with the phenyl-hydrazine test a crystalline p h e n y l - l a c t o s a z o n e, somewhat resembling the analogous compound given by dextrose, but the crystals are much broader than those of phenyl-glucosazone, melt at 200° C. instead of at 204°, and dissolve more readily in alcohol.

Milk-sugar reduces alkaline solutions of copper, and has a specific rotation of +55 8° for the sodium ray

When boiled with dilute mineral acids (citric acid has no action) milk-sugar is split up into two glucoses, dextrose and galactose The latter has been identified with the sugar obtained by Thudichum by the action of dilute sulphuric acid on certain constituents of the brain, and called by him c e r e b r o s e

Researches by F. W P a v y (*Physiology of the Carbohydrates*, 1894) also appear to show that a reducing sugar analogous to, if not identical with, galactose is produced by the direct treatment of proteid matters with dilute sulphuric acid After this treatment the product is nearly neutralised with baryta, and the neutralisation completed with barium carbonate. The filtered liquid is concentrated, and heated on the water-bath for at least an hour with acetic acid and phenyl-hydrazine (or phenyl-hydrazine hydrochloride and sodium acetate) Crystals of an osazone occasionally make their appearance in the hot liquid, but more frequently are deposited during and after cooling From the crystalline osazone thus obtained the sugar itself is susceptible of recovery by the process described by Fischer The "sugar" thus obtained gives a clear and definite reaction with Fehling's solution, attended with distinct deposition of red cuprous oxide, and it yields a crystalline osazone melting at 189° to 190° C [1] It is, however, optically inactive, and unfermentable with yeast

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[1] Galactosazone melts at 193° to 195° C

MALTOSE, $C_{12}H_{22}O_{11}$, is said to have been found in diabetic urine by Le Nobel It is dextro-rotatory ($\alpha_D = +138\ 9°$), reduces alkaline solutions of copper, &c ; and yields with phenyl-hydrazine a compound which crystallises in yellow tables melting at 82° C

SUCROSE or CANE-SUGAR, $C_{12}H_{22}O_{11}$, is alleged to be liable to occur in the urine of persons who consume large quantities of sugar It is dextro-rotatory ($\alpha_D = +66\ 5°$), does not reduce alkaline cupric solutions, and forms no compound with phenyl-hydrazine

DEXTRIN, $C_6H_{10}O_5$ In some cases of diabetes in which the sugar gradually disappeared, R e i c h a r d (*Pharm Zeitschrift fur Russland*, xiv 45) found Fehling's solution to become gradually green, then yellow, and sometimes finally dark brown He suspected this to be due to the presence of *dextrin*, which he succeeded in isolating by the following process The urine was evaporated to a syrup, absolute alcohol and caustic potash added, the precipitate allowed to settle, and the liquid decanted The precipitate was washed with absolute alcohol, and then dissolved in acetic acid On repeated addition of absolute alcohol to this solution the dextrin was deposited as a white substance, which after drying was tasteless, gave the dextrin reaction with Fehling's test, a brown coloration with iodine solution, and was readily converted into glucose by boiling with dilute sulphuric acid

ANIMAL GUM is a carbohydrate obtained by L a n d w e h r by the action of hydrochloric acid on mucin, and said to occur in small quantities in urine It forms an opalescent solution in water, the resultant liquid giving a sticky precipitate with ferric chloride and cupric sulphate solutions It does not reduce alkaline cupric solutions until after boiling with dilute sulphuric acid Landwehr's body yields oxalic acid

on oxidation with nitric acid, and lævulic acid by treatment with hydrochloric acid   It does not ferment with yeast, is precipitated by alcohol, and gives no coloration with iodine

GLYCOGEN, $(C_6H_{10}O_5)_n$, has been termed " animal starch," and presents the closest analogy to soluble starch   It was first found in the liver, but has more recently been met with in many other parts of the body

Pure glycogen is a white, amorphous powder, readily soluble in water to form a solution which is usually, but not invariably, opalescent, and becomes more limpid on adding acetic acid or an alkali. Glycogen is precipitated from its aqueous solution by alcohol whenever the alcohol amounts to 60 per cent of the liquid   If the solution be quite free from salts, the separation is sometimes very difficult, but takes place instantly on adding a minute quantity of common salt   The precipitation of glycogen in liquids containing 60 per cent of alcohol distinguishes it from the different varieties of dextrin, none of which are precipitated by alcohol of less than 85 per cent strength   Glycogen exactly simulates dextrin in its behaviour with a solution of iodine, which produces a port-wine colour, disappearing on heating, and returning as the liquid cools

Glycogen is strongly dextro-rotatory, the value of $[\alpha]_D$ varying from $+203°$ to $+234°$, according to the concentration of the solution

Glycogen does not reduce Fehling's solution   It is precipitated by baryta-water as $BaO(C_6H_{10}O_6)_3$, and by basic lead acetate as $PbO(C_6H_{10}O_6)_2$

When boiled with dilute nitric acid, glycogen yields oxalic acid   Boiled with dilute sulphuric or hydrochloric acid, it is converted into dextrose   It does not ferment with yeast, but diastase and saliva convert it

into maltose and achroo-dextrin, a little dextrose being also formed   On the other hand, in the hydrolysis of glycogen in the liver, dextrose and not maltose is the chief product

Glycogen is best prepared by rapidly cutting up the liver of an animal killed immediately previously, and throwing the fragments into five times their weight of boiling water   After boiling for a short time, the fragments of liver are mixed with sand and reduced to powder in a mortar, and then returned to the water, which is again boiled   The liquid is strained, and faintly acidified with acetic acid while still hot The filtrate from the coagulated proteids is rapidly cooled, and the remaining proteids precipitated by the alternate addition of hydrochloric acid and potassio-mercuric iodide   The filtered liquid is mixed with such a volume of strong spirit as to make it contain 60 per cent of absolute alcohol, when the precipitated glycogen is filtered off, washed first with 60 per cent spirit, and then with absolute alcohol and ether [1]

The researches of Claude Bernard first established the fact of the formation of a substance in the liver which, from the circumstance of its ready conversion into glucose, he termed "glycogen"   Bernard

[1] Bräutigam and Edelmann (*Chem Centr*, 1894, i 485, *Jour Chem Soc*, lxvi ii 336) have proposed to distinguish horse flesh from the flesh of other animals by a reaction based on its high content of glycogen Ten per cent of horse flesh or liver can be detected by this means, the proportion present ranging from 0 37 to 1 07 per cent, while the flesh of other animals used for food contains little or none—ox flesh coming next with 0 20 per cent   The finely divided flesh is boiled with four times its weight of water, and the resulting broth treated with dilute nitric acid to precipitate albumin, and filtered   Saturated hydriodic acid is now added, so the two liquids may remain as distinct layers, when a red or violet zone will be seen at the junction of the two strata if glycogen be present   The reaction is said to be quite characteristic of horse flesh   In the event of the extraction of the glycogen with water proving inadequate, a solution of caustic potash containing KOH equal to 3 per cent of the weight of the flesh must be substituted   Humbert substitutes a saturated solution of iodine in boiling water for the hydriodic acid (*Jour Pharm et Chim*, 1895, 195, *Analyst*, xx 95)

affirmed, as the result of his experiments, that glycogen is normally, and as a physiological action of the liver regularly, transformed into glucose, that in health, this glucose, after being carried into the circulation by the hepatic veins, is destroyed by oxidation, but that in certain abnormal conditions this natural transformation is arrested   These views of Bernard have been strongly combated by P a v y and others, according to whom sugar is not found in the liver when examined instantly after death, and is not present in any quantity in the blood of the right side of the heart, as it would be if sugar were constantly being formed in the liver and conveyed away from that organ by the hepatic vein [1]

Researches by V o i t, O t t o, A b b o t t, L u s k, and others render it probable that the formation of glycogen in the liver may arise in two ways as a temporary store of carbohydrate food, and as the result of proteid metabolism   Doses of dextrose, lævulose, cane-sugar, and maltose increased the hepatic glycogen, while lactose and galactose did not, or only very slightly, but both these sugars increased the proportion of sugar eliminated in diabetes   In the alimentary canal, cane-sugar and maltose undergo inversion   Lævulose, lactose, and galactose are absorbed as such, and after large doses pass unchanged into the urine   The liver appears unable to form glycogen from sugars which, like lactose and galactose, do not pass into the condition of either dextrose or lævulose. Dextrose and lævulose appear to be the only kinds of sugar which, when present in the blood, lead to the storage of glycogen in the liver, and the liver-cells

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[1] The blood in the right side of the heart consists of the venous blood returned from the whole body, *plus* that returned from the liver by the hepatic vein   The amount of sugar in the blood of the hepatic vein is the datum requisite for completing the argument

either convert lævulose into glycogen direct, or after previously changing it to dextrose

If carbohydrates be introduced into the stomach or blood of rabbits, the liver of which has been rendered quite free from glycogen by six days' starvation, a large amount of glycogen will be found in the liver after a few hours

It is probable that the glycogen stored in the liver and the muscles is not derived exclusively from the carbohydrates of the food   It appears that the albuminous and gelatinous constituents of the food are also concerned in its formation   Thus animals which have been fed wholly on lean meat for a considerable period exhibit large stores of glycogen in their liver and muscles   In the severer forms of diabetes, under a protracted and exclusive flesh diet, the secretion of sugar does not cease, and even increases in proportion to the amount of albumin consumed

V o n M e r i n g found that when phloridzin, a glucoside contained in the root-bark of cherry and apple trees, was administered to a dog, sugar appeared in the urine after a few hours   This glycosuria ceased in a few days, and the liver and muscles are then wholly free from glycogen   On again administering phloridzin, a still larger amount of sugar was excreted Unless the sugar was produced from proteids, it must be assumed to have been formed from fat, for which assumption there is no foundation

INOSITE, $C_6H_{12}O_6$, sometimes improperly called "muscle-sugar," has been met with in healthy urine after the use of diuretics or a large excess of water. D a h n h a r d t isolated 0 1 gramme of inosite from 8 litres of ox urine

Inosite was found by G a l l o i s in the urine of five diabetic persons out of a total of thirty examined.

Five of these samples contained sugar in various proportions in addition to mosite. Inosite has also been found in the urine in cases of albuminuria, syphilis, phthisis, and typhus fever

For the detection of mosite, several litres of the urine should be treated with excess of neutral lead acetate, filtered, and the filtrate warmed and treated with basic lead acetate till precipitation is complete The liquid is filtered after standing forty-eight hours, and the washed precipitate suspended in water and decomposed with sulphuretted hydrogen The filtrate deposits uric acid on standing, when it is again filtered, evaporated to a syrup at 100°, and treated with absolute alcohol The precipitate is dissolved in hot water, and three or four volumes of rectified spirit added. Ether is then gradually added until a permanent turbidity is produced, when the mosite crystallises on standing

Pure mosite crystallises in rhombic prisms sometimes grouped in rosettes, or, from its solution in hot rectified spirit, in shining scales, or in tables which, under the microscope, somewhat resemble cholesterin (fig 2) The
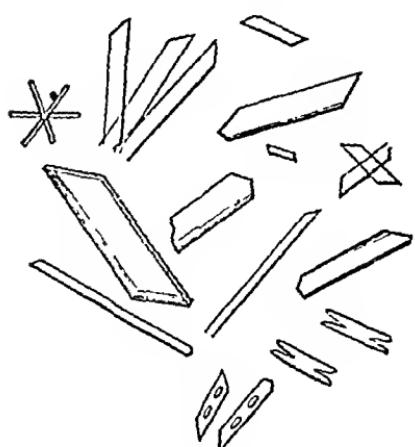
Fig 2 —INOSITE

crystals easily effloresce, and sometimes turn red When impure, mosite is often obtained in mammillated forms It dissolves in 16 parts of cold water, and is soluble in rectified spirit, especially when hot, but is insoluble in absolute alcohol or ether Inosite melts at 210° C, has a sweet taste, is optically inactive, and does not ferment with yeast, but by treatment with putrid albumen it is said to

yield sarcolactic acid   Inosite does not reduce Fehling's solution   It is not precipitated by neutral lead acetate, but is precipitated by the basic acetate, especially on heating, as the compound $C_6H_{12}O_6,2PbO$ It yields no osazone with phenyl-hydrazine [1]

According to G a l l o i s, if an aqueous solution of inosite be treated in porcelain with a drop of solution of mercuric nitrate a yellowish precipitate will form, which, if spread out on the edge of the dish quickly and heated carefully, becomes dark red   On cooling, the colour disappears, but is reproduced on again heating   The reaction is not produced by glycogen, starch, mannite, milk-sugar, urea, uric acid, glycocine, taurin, or cystin   Albumin assumes a rose colour, and sugar becomes black under similar treatment

According to S c h e r e r (*Ann Chem. und Pharm*, 1852, page 375), if a solution of inosite be evaporated nearly to dryness at 100° with a few drops of nitric acid, and the nearly dry product moistened with ammonia and calcium chloride solution, a bright pink or rose-red coloration is obtained on completing the evaporation

GLYCURONIC ACID $C_6H_{10}O_7$, or $COH(CH\,OH)_4\,COOH$ Glycuronic acid doubtless has its origin in the d e x t r o s e of the body, to which compound it is closely related [2]   It was first obtained in the conjugated form of c a m p h o - g l y c u r o n i c a c i d in the

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[1] Although having the empirical formula of a glocuse, inosite is not a true carbohydrate   It really belongs to the aromatic series, and may be represented thus $-CH(OH)\left\{\begin{array}{l} CH(OH)\ CH(OH) \\ CH(OH)\ CH(OH) \end{array}\right\}CH(OH)$

[2] The relation between glycuronic acid and bodies of the sugar group is shown by the following constitutional formulæ —

Dextrose,	$CH_2(OH)\,(CH\,OH)_4\,CO\,H$
Gluconic acid,	$CH_2(OH)\,(CH\,OH)_4\,CO\,OH$
Saccharic acid,	$CO(OH)\,(CH\,OH)_4\,CO\,OH$
Glycuronic acid,	$CO(OH)\,(CH\,OH)_4\,CO\,H$
Gulonic acid,	$CO(OH)\,(CH\,OH)_4\,CH_2\,OH$
Gulose,	$CO(H)\,(CH\,OH)_4\,CH_2\,OH$

urine of dogs to which camphor had been administered, and subsequently as u r o - c h l o r a l i c a c i d after the administration of chloral   It is remarkable for its tendency to form ethereal or glucosidal compounds when appropriate substances are introduced into the body.   Traces of such compounds probably occur normally in urine, especially i n d o x y l- and s k a t o x y l-g l y c u r o n i c a c i d s , in addition to the combination with urea, having probably the constitution of u r o-g l y c u r o n i c a c i d, which appears to be the ordinary form in which glycuronic acid exists in urine

B a e y e r (*Annalen*, clv 257) has shown that e u x a n t h i c a c i d, which exists in combination with magnesia in the " purrée " or " Indian yellow " of commerce,[1] is decomposed on boiling with hydro-

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[1] *Puri* or *Purrée*, now used as a pigment under the name of " Indian yellow," is obtained in Bengal from the urine of cows which are fed exclusively on the leaves of the mango tree and water   The urine is heated, and the precipitate separated and dried   Analyses of very pure specimens of purrée by C G r a e b e (*Annalen*, cchv 265) showed   euxanthic acid, 51 , silica and alumina, 1 5 , magnesia, 4 2 , lime, 3 4 , and water and volatile substances, 39 per cent   The analyses of Stenhouse and Erdmann show much less lime Urea, uric acid, and hippuric acid have also been found in purree   The poorer qualities contain considerable quantities of e u x a n t h o n e, partly free and partly in combination   For the isolation of the euxanthic acid and euxanthone, and the assay of purrée, the colouring matter should be triturated with dilute hydrochloric acid until the whole has assumed the bright yellow colour of euxanthic acid   The residue is then well washed with cold water to remove the salts, and the euxanthic acid extracted from the residue by ammonium carbonate solution   It is precipitated from the filtrate by hydrochloric acid, and purified by crystallisation from alcohol   The euxanthone, left undissolved by the ammonium carbonate, is treated with caustic soda, the solution precipitated with an acid, and the precipitated euxanthone shaken out with ether or filtered off and dried at 100°

Euxanthic acid has the constitution —

$$\text{OH } C_6H_3 \left\{ \begin{matrix} O \\ CO \end{matrix} \right\} C_6H_3 \text{ O CH(OH) (CH OH)}_4 \text{ COOH}$$
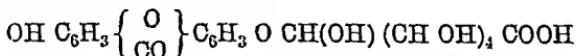
It forms pale yellow needles, which melt at 156–158°   It has a sweet taste and bitter after taste, is but slightly soluble in cold water, very sparingly in ether, but readily in boiling alcohol   Alkalies colour the solution deep yellow Euxanthic acid does not reduce Fehling's solution, nor form a compound with phenyl hydrazine

Euxanthone is a neutral substance, crystallising in pale yellow needles, soluble in alkalies but not in dilute acids   It forms no compound with phenyl-hydrazine

chloric acid or dilute sulphuric acid, with formation of
e u x a n t h o n e and an acid which has been shown by
S p i e g e l (*Ber*, xv 1965) to be identical with g l y-
c u r o n i c a c i d, $C_{19}H_{18}O_{11} = C_{13}H_8O_4 + C_6H_{10}O_7$ In
fact purrée is the best material for the preparation
of glycuronic acid, which can be obtained on the small
scale by the following process —The artists' water-
colour known as " Indian yellow " is ground up with
sand, and then treated with dilute hydrochloric acid,
which dissolves out calcium and magnesium salts, &c
The residue is washed with water and treated with a
solution of ammonium carbonate, which dissolves the
euxanthic acid, leaving euxanthone and sand undis-
solved   From the filtered liquid the euxanthic acid
is precipitated by dilute hydrochloric acid, washed
with cold water, and then heated with water in a
closed soda-water bottle to 125° C for three or four
hours   The requisite temperature can be conveniently
obtained by immersing the bottle in a bath of molten
paraffin wax (candles)   From the cooled product the
euxanthone is dissolved by agitation with ether, and
the glycuronic anhydride crystallised from the concen-
trated aqueous liquid.

Glycuronic acid is a syrupy liquid, miscible with water
or alcohol   When the aqueous solution is boiled, eva-
porated, or even allowed to stand at the ordinary
temperature, the acid loses the elements of water and
yields the a n h y d r i d e or l a c t o n e

Glycuronic Anhydride, $C_6H_8O_6$, forms monoclinic
tables or needles, having a sweet taste, and melting
at about 160° when heat is gradually applied, or at
170–180° when heated rapidly.   The anhydride is in-
soluble in alcohol, but dissolves readily in water to form
a dextro-rotatory solution $[\alpha_D] = 19\ 25°$ The solu-
tion prevents the precipitation of cupric solutions by
alkalies, and powerfully reduces hot Fehling's solution,

the cupric oxide reducing power being 98 8, compared
with glucose as 100   On distillation with hydro-
chloric acid, glycuronic anhydride (as also uro-chloralic
acid) yields furfuraldehyde, a trace of which substance
is also obtainable on similarly treating normal urine

Glycuronic acid itself is dextro-rotatory ($[a]_D = +35°$),
but many of its compounds are lævo-rotatory[1]   It
reduces Fehling's solution on heating, and precipitates
the metals from hot alkaline solutions of silver, mercury,
and bismuth

On oxidation, glycuronic acid yields camphoric and
formic acids   By treatment with bromine it yields
s a c c h a r i c   a c i d, $C_6H_{10}O_8$, a reaction which in-
dicates the presence of an aldehyde group and the
close relation between glycuronic acid and dextrose
Saccharic acid can again be reduced to glycuronic acid
by treatment with sodium amalgam, further treatment
yielding   g u l o n i c   a c i d, $C_6H_{12}O_7$, a body which
does not reduce Fehling's solution   (F i s c h e r   and
P i l o t y, *Ber*, xxiv 521 , *Jour Chem Soc*, lx 667)

When boiled with caustic alkali, glycuronic acid
yields oxalic acid as an invariable product   Catechol
and protocatechuic acid are also formed if concentrated
alkali be employed for the treatment

Glycuronic acid is distinguished from glucose by not
undergoing the alcoholic fermentation when treated
with yeast   On the other hand, when fermented in
presence of cheese and chalk it yields lactic and acetic
acids

Glycuronic acid forms a potassium salt which
crystallises in needles   The sodium salt is similar
The zinc, cadmium, copper, silver, and calcium salts
are uncrystallisable   The barium salt is amorphous

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[1] After taking chloral hydrate the urine contains trichlorethyl glycuronic
acid (" uro chloralic acid "), a lævo rotatory body which is decomposed into
trichlorethyl alcohol and dextro rotatory glycuronic acid

and soluble in water It is the compound employed for the isolation of glycuronic acid from urine

With phenyl-hydrazine, glycuronic acid forms a yellow crystalline compound melting at 114° to 115° C , but under modified conditions an amorphous, brownish-yellow body, melting at 150° C , is produced According to J. A Hirschl, normal urine yields this compound (see page 88)

For the actual isolation of glycuronic acid from urine a large quantity of the excretion is required The method is described in an interesting report by H H Ashdown (*Brit Med Jour* , 1890, i 169, and *Pharm Jour* , [3], xx 607)

Glycuronic acid occurs in the urine to a very notable extent after the administration of morphine, chloroform, chloral, butyl-chloral, nitrobenzene, camphor, curare, and certain other drugs It was undoubtedly mistaken for glucose by the older observers In one case recorded by Ashdown large amounts of glycuronic acid occurred in the urine of a healthy young man, whose excretion was not abnormal either in volume or density

GLYCOSURIC ACID is a body extracted by J Marshall from pathological urine (*Arch Pharm* , [3], xxv 593, and *Jour Chem Soc* , liii 1047) It is isolated by a process based on the insolubility of the lead salt in alcohol of 45 per cent Glycosuric acid crystallises in opaque tetragonal prisms, which melt at 140°, are readily soluble in water, alcohol, and ether, less readily in chloroform, and insoluble in benzene, toluene, and petroleum spirit Glycosuric acid contains no nitrogen, is readily and completely absorbed by animal charcoal, and appears to be a phenolic derivative It reduces Fehling's solution more powerfully than glucose, and also reduces silver nitrate , but not bismuth compounds or alkaline solutions of picric acid

It gives a brown coloration when boiled with aqueous soda A dilute and neutral solution of ferric chloride gives an evanescent blue colour On evaporating the ethereal solution of glycosuric acid at 60° the liquid takes a wine-red colour, which is imparted to the crystals which separate, but these form a colourless solution in water

A Geyger (*Pharm Zeit*, 1892, page 1488) extracted glycosuric acid from a diabetic urine by acidulating it with sulphuric acid and agitating with ether The ether left on evaporation a crystalline substance, melting at 143° C, which proved to be the acid in question He suggests that diabetic urine should always be examined in this manner

DETECTION AND DETERMINATION OF SUGAR IN URINE.

A great variety of methods have been proposed for the recognition and estimation of sugar in urine Many derive the only value they possess from being simple in application, and therefore not beyond the manipulative skill and appliances of the general medical practitioner Not a few are utterly worthless and misleading in their indications, while others fail to show the presence of proportions of sugar (*e g* , below $\frac{1}{2}$ per cent) which are not without grave pathological significance, and of the greatest importance as a warning to the patient

With the exception of a few colour-reactions of little value, the processes for detecting and determining sugar in urine may be arranged in the following classes —

1 Isolation of the dextrose as such

2 Methods based on the fermentation of the glucose.

3 Methods based on the optical activity of dextrose

4 Methods based on the reducing action of glucose
These include the various modifications of the copper, mercury, and bismuth tests, and the colour-changes produced by glucose in hot alkaline solutions of various coal-tar colours

5 Methods dependent on the conversion of the sugar into definite crystalline compounds, such as those yielded with benzoyl chloride and phenyl-hydrazine

Isolation of Sugar from the Urine

When typical diabetic urine, containing from 5 to 10 per cent of glucose, is in question, it is not difficult to isolate the dextrose in a pure state   On evaporating such urine on the water-bath to the consistence of a syrup, and then leaving it at rest for some days, dextrose will frequently separate in tabular crystals or irregular warty masses   More frequently it is deposited in crystals of a compound of dextrose with sodium chloride containing $2C_6H_{12}O_6,NaCl+H_2O$, which is less soluble in alcohol but more soluble in water than glucose itself   Not unfrequently the syrup may remain for months without any crystals appearing   Sometimes treatment of the syrup with ether, which is subsequently allowed to evaporate spontaneously, facilitates the formation of crystals   The syrup, with any crystals contained in it, is treated with cold absolute alcohol, which dissolves out urea and extractive matter, but leaves most of the glucose   On boiling the residue with rectified spirit the sugar is dissolved, sulphates, phosphates, urates, &c , remaining insoluble   The liquid is filtered hot and evaporated to a small bulk, when it will deposit crystals of glucose on cooling , these may be purified by recrystallisation from boiling rectified spirit

For the isolation of the minute quantity of sugar existing in non-diabetic urine, F W P a v y employs the following adaptation of a process devised by B r u c k e —A large quantity of the urine is treated with half its measure of a 10 per cent solution of neutral lead acetate   The precipitate, which contains urates, sulphates, phosphates, colouring matter, &c , is filtered off, and the filtrate treated with basic lead acetate, any further precipitate being removed as before   The filtered liquid is then treated with ammonia and more lead acetate, unless a distinct excess has been already used   This causes the precipitation of the glucose as a definite compound with lead oxide   The precipitate is filtered off and washed with cold water till the greater part of the ammonia is removed, when it is suspended in water and decomposed by a stream of sulphuretted hydrogen Sulphuric or oxalic acid may be substituted for the last reagent, but the product is more strongly coloured In any case, the strongly acid liquid should be treated cautiously with sodium carbonate solution till neutral, when colouring matter will separate and may be filtered off   Further decolorisation may be effected by treating the liquid, rendered slightly acid with acetic acid, with a little animal charcoal (previously carefully purified from phosphates, &c , by boiling with hydrochloric acid and washing till free from acid reaction to litmus)

From this clarified liquid, dextrose can be obtained in crystals recognisable under the microscope, but for its identification by chemical tests the solution itself is quite suitable   Working on the product from healthy persons, Pavy obtained all the chief reactions of sugar, including the production of carbonic acid gas by fermentation with yeast and the formation of a crystalline compound with phenyl-hydrazine

Pavy's results go far to decide in the affirmative the long-vexed question of the existence of traces of sugar in normal human urine (compare, however, page 20)

Fermentation Test for Sugar

The recognition of sugar in urine, by its conversion into alcohol and carbon dioxide when treated with yeast, has the advantage of simplicity and almost absolute certainty that the effect produced is really due to sugar   On the other hand, the experiment extends over a comparatively long time, and the method is deficient in delicacy and accuracy

In its simplest form, the fermentation test is applied by introducing a fragment of pressed yeast the size of a nut into a 4-oz bottle, filling the bottle completely with the urine to be tested, and then inverting the vessel over a small capsule or saucer of the urine, in such a manner as not to allow any air to enter   The whole is then left in a warm place—from 25° to 30° C (= 77° to 86° F)—for twelve or fifteen hours, when, if sugar be present in quantity, the liquid will become lighter in colour, and diminish in gravity, while at the same time a notable quantity of carbon dioxide will collect in the upper part of the bottle The test fails, or answers but imperfectly, when the proportion of sugar is less than 0 5 per cent   By keeping the bottle in an erect position, and allowing the fermentation to continue for twenty-four hours, a good approximation to the content of sugar may be obtained by noting the decrease in the density of the liquid   As pointed out by Sir W Roberts, every degree of gravity lost by the fermentation corresponds closely with 1 grain of sugar per fluid ounce of the urine   Another portion of the same urine, untreated with yeast, should be kept side by side with the

fermentation test, and its density observed under similar conditions to the latter   Thus, if the untreated urine be found to have a gravity of 1042, and the portion treated with yeast have on completion of the fermentation a density of 1015, the urine will have contained 27 grains of sugar per fluid ounce (1042 − 1015 = 27)   Attempts have been made to obtain more accurate results by this process, but the method has too many sources of error to make such refinements of practical value

Instead of observing the diminution in density, the proportion of sugar may be deduced from the weight or measure of carbon dioxide (carbonic acid) gas evolved Many ways of effecting this have been devised, but one of the simplest and most effective is to introduce a known measure of the urine to be tested into a bottle furnished with a perforated cork, and attached by elastic tubing to the three-way tap of a nitrometer filled with brine (see "Urea")   Yeast is added to the urine, and the arrangement left for twenty-four hours in a warm place, as previously described   The gas which has collected in the nitrometer is then measured with the usual precautions   One c c of CO_2, measured over brine at the ordinary pressure and temperature (about 30 inches of barometric pressure, and at 15° to 20° C , = 59° to 69° F), represents 0 002 gramme of glucose in the urine taken

Instead of using a separate vessel for the urine, 5 c c may be introduced, through the tap, into a nitrometer filled with mercury, 5 c c of water containing as much yeast as possible in suspension run in through the tap, and the apparatus kept in a warm place until the fermentation is complete

Where a nitrometer and the other appliances of a laboratory are not available, a very fair determination of the sugar in diabetic urine may be made by the

arrangement shown in fig 3, constructed from a feeding-bottle and ordinary wash-bottle   The glass tube is removed from the inside of the feeding-bottle, and the india-rubber tube fixed air-tight into the stopper by means of sealing-wax   The nipple is removed from the other end of the india-rubber tube, which is then slipped on to the mouth-tube of an ordinary wash-bottle   Three fluid drachms ($= \frac{3}{8}$ oz) of the urine to be tested, or an equivalent measure of the diluted sample,[1] should then be placed in the feeding-bottle, about an ounce of water and a piece of pressed yeast added, and the stopper with its attached tubes firmly adjusted   The wash-bottle is filled with water, or preferably with brine, and its cork (carrying the tubes) firmly fixed in   This will cause the exit tube to fill with water. A suitable measuring vessel, such as a graduated 8-oz medicine-bottle, is placed below the outlet tube so
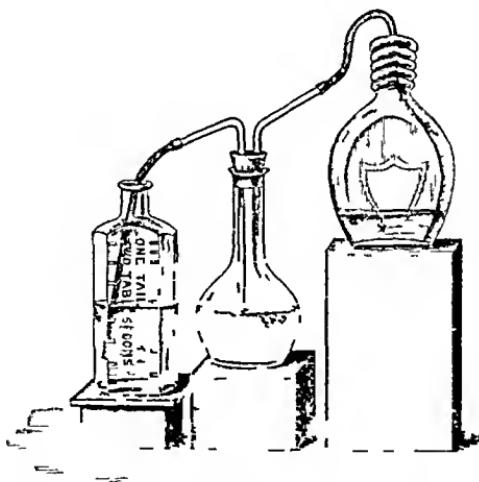
Fig 3 —Apparatus for estimating diabetic sugar by fermentation

as to receive the brine displaced by the gas generated by the fermentation   The whole arrangement is kept at a temperature of from 25° to 30° C (= 77° to 86° F) for twenty-four hours, when the volume of

[1] 1 oz of the sample may be diluted with 3 oz of water, and $1\frac{1}{2}$ oz of the diluted liquid ($= \frac{3}{8}$ oz of the original sample) taken for the experiment

liquid collected in the measuring vessel is observed as accurately as possible.

In any experiment where it is sought to determine sugar by fermentation, it is of course essential that the yeast employed be free from starch. Much of the pressed yeast met with in commerce contains a considerable proportion of starch, which is said to be added as a preservative, but is certainly not essential. The presence of starch may be readily detected by mixing up a little of the yeast with tincture of iodine, which will produce a blue or violet colour if starch be present.

If $\frac{3}{8}$ ounce (= 3 fluid drachms) was the measure of urine used for the experiment, every ounce of fluid displaced will represent 1 per cent of sugar in the sample; while every 100 grains measure will correspond to 1 grain per fluid ounce of the urine [1]

Fresh brewers' yeast may be substituted for pressed yeast if it be first well washed on a filter with cold water.

In all the foregoing modifications of the fermentation test it is important that the urine should be moderately but distinctly acid. The safest plan is to render the liquid slightly alkaline to litmus paper, to ensure the neutralisation of any possible mineral acid, and then to acidify the liquid with tartaric acid to the extent of about 0 5 gramme per 100 c c (2 grains per fluid ounce)

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[1] The main reaction occurring in the fermentation of glucose is expressed by the equation $C_6H_{12}O_6 = 2C_2H_6O + 2CO_2$. Hence 180 parts of sugar yield 92 of alcohol and 88 of carbon dioxide. (This statement is not strictly correct, but sufficiently so for the purpose in question.) 88 grammes measure 44,760 c c at the standard pressure and temperature, or about 48,000 c c at 20° C (= 68° F). Hence this measure of gas will be yielded by 80 grammes of glucose, 800 by 3 grammes, or 800 grains measure by 3 grains. If the urine contain 1 grain of glucose per fluid ounce, and $\frac{3}{8}$ oz is employed, the liberated gas will measure $\frac{800}{3} \times \frac{3}{8} = 100$ grains measure. But if the content of sugar be 1 per cent, or 4 375 grains per fluid ounce, the gas will measure $437\frac{1}{2}$ grains, or exactly 1 oz. If 15 c c of urine be taken, then every 10 c c of liquid displaced by the gas represents $\frac{1}{4}$ per cent of sugar in the sample

Polarimetric Determination of Diabetic Sugar.

The sugar contained in diabetic urine is commonly assumed to be identical with the dextro-glucose present in grapes and produced, together with lævulose, by the inversion of cane-sugar   It is not quite certain, however, that this assumption is correct, nor that the dextrose is not sometimes associated with lævulose or some other kind of sugar   The subject requires further investigation by competent observers, who will require to determine the total sugar by some more certain process than those commonly used for the purpose, comparing the results so obtained with those yielded by the optical method   In ordinary cases, however, the polarimeter can be trusted to give excellent comparative, if not rigidly accurate, results, provided the proportion of sugar is not too small   For the detection or determination of the traces of sugar supposed to be present in healthy urine, or even for the examination of slightly diabetic urine, the optical method is useless, since urine ordinarily contains traces of various substances other than sugar which are more or less optically active [1]

The proportion of glucose in urine can be deduced from the following formula, in which c is the *concentration*, or grammes of sugar per 100 c c of urine

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[1] M Carles (*Jour de Pharm* , 1890, page 108) states that all normal urines have a lævo rotation varying from 0 25 to 0 80° of the saccharometer, the precise activity varying with the proportion of extractive matter, intensity of colour and odour, amount of urea, and specific gravity   Carles inclines to the opinion that the optical activity of normal urine is due, at least in part, to the creatinine   He describes a urine which contained 33 grammes of urea and 2 27 of phosphoric acid per litre, and had a specific gravity of 1 025, which exhibited a left handed rotation of 1 93°   Hence it might have contained 4 35 grammes of sugar per litre, and yet have been without dextro rotation

According to Haas, dextrose is the chief, and almost the only constituent of urine producing dextro rotation, while lævo rotation may be due to glycuronic acid, its phenyl and indoxyl derivatives, leucine, tyrosine, albumin, hemialbumoses, peptones, cystin, cholesterin, aspartic acid, pseudo butyric acid, and lævulose   In addition may be mentioned the disturbing influence of quinine and other alkaloids

D

a the *angular rotation* observed in the polarimeter, and *l* the *length* of the tube in decimetres,

$$c = \frac{a \times 100}{l \times 52\ 7°}$$

This equation applies only to instruments in which monochromatic sodium light is employed With saccharometers intended for observations of the transition-tint, 57 0° must be substituted for 52 7°

In cases where the optical method may be suitably employed, its application is very simple [1] Diabetic urine is usually so pale in colour that its rotation may be observed without previous clarification If the colouring matter of the sample should occasion any difficulty in the observation, simple agitation of 100 c c of the cold urine with about 3 grammes of neutral acetate of lead, and filtration through paper, will generally produce an almost water-white filtrate

Detection and Determination of Diabetic Sugar by its Reducing Action.

The methods of examining urine for glucose most generally employed are those based on its reducing action, which is powerfully exerted in boiling alkaline solutions Thus the sugar of diabetic urine, which is chiefly if not entirely dextrose, *in hot alkaline solutions* effects the following reductions The oxygen shown in the reactions is not evolved as such, but converts the glucose into gluconic acid and other oxidation-products

(*a*) *Cupric oxide*, retained in solution by fixed organic matter, ammonia, or cyanides, is reduced to cuprous oxide —$2CuO = Cu_2O + O$

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[1] The method of using the polarimeter is familiar to those accustomed to employ the instrument, and is described in great detail in various works readily accessible A Laurent instrument on the half shadow principle is to be preferred to other forms A polarimeter of this kind, obtainable at a moderate price, is described in *The Analyst*, vol xi page 141 Another useful instrument is made by Messrs Philip Harris & Co , Birmingham

(b) *Bismuthic oxide*, in the solid state, or in alkaline citrate solution, is reduced to metallic bismuth — $Bi_2O_3 = Bi_2 + O_3$

(c) *Mercuric oxide*, in the form of potassio-mercuric cyanide or iodide, and in presence of caustic alkali, is reduced to metallic mercury — $HgO = Hg + O$

(d) *Orthonitrophenyl-propiolic acid* is reduced to indigo-blue — $2C_9H_5NO_4 = C_{16}H_{10}N_2O_2 + 2CO_2 + O_2$
Indigo-blue is, by further treatment, reduced to white indigo — $C_{16}H_{10}N_2O_2 + H_2O = C_{16}H_{12}N_2O_2 + O$

(e) *Ferricyanides*, in presence of fixed caustic alkali, are reduced to ferrocyanides — $2K_3(C_6N_6Fe) + 2KHO = 2K_4(C_6N_6Fe) + H_2O + O$

(f) *Picric acid* is reduced to picramic acid — $C_6H_2(NO_2)_3 OH + 3H_2O = C_6H_2(NH_2)_3 OH \quad O_9$

(g) *Methylene-blue* is converted into the corresponding leuco-compound — $C_{16}H_{19}N_3SO = C_{16}H_{19}N_3SO$

(h) *Safranine* is similarly reduced to the corresponding leuco-compound

Some of the foregoing decompositions take place at the ordinary temperature, or considerably below the boiling-point, if time be allowed for the reaction

The reaction which occurs when a solution of glucose is heated with an oxidising agent in presence of caustic alkali is complex, and has been but imperfectly studied The principal products are said to be formic, oxalic, glycollic and carbonic acids, but the products of the action of the alkali itself on the sugar have evidently to be taken into account (compare page 24) The practical necessity of employing caustic alkali in conjunction with the oxidising agent is a grave defect of all quantitative tests based on the reducing action of glucose

The recognition of glucose in urine, by its reducing action on the foregoing and similar yielders of oxygen, is readily effected when the proportion of reducing

sugar is considerable, but, when very small quantities are in question, the indications of the reagents are not to be depended on   This is not so much due to any deficiency in the delicacy of the reactions, as a consequence of the presence in urine of other bodies which resemble glucose in their power of exercising more or less reducing action on oxidising agents   Under abnormal conditions urine may contain many such reducing bodies, but even normal urine invariably contains traces of some of such bodies, and under special conditions of diet, or under the influence of medicines, these and others may be increased to a very material extent

Of the constituents of normal urine which resemble sugar in their reducing action, the most important are uric acid and creatinine, but hippuric acid, creatine, and glycuronic acid and its compounds exercise a similar action, though their quantity is usually so insignificant that they may safely be neglected   Very little is known of the specific action of these urinary bodies on the before-mentioned oxidising reagents, and of their quantitative behaviour we know still less   The alkaline solutions of copper are almost the only reagents of which the oxidising power on urinary constituents other than glucose is even approximately known, and the published statements stand in much need of verification and revision   It may, however, be taken as an established fact that creatinine, uric acid, and glycuronic acid and its compounds reduce both Fehling's and Pavy's copper solutions   Creatinine also reduces an alkaline solution of picric acid, with production of a blood-red coloration, but uric acid does not reduce picric acid even on boiling   Safranine in alkaline solution is reduced to a leuco-compound by glucose, but not by creatine, creatinine, uric acid, or hippuric acid

The following table shows the behaviour of various precipitants and alkaline oxidising agents when heated to boiling with the chief urinary constituents

Reagent	Albumin	Glucose	Uric Acid	Creatinine	Xanthine	Remarks
Neutral lead acetate	Ppted	Not affected	Ppted	Not affected	Ppted	
Basic lead acetate	Ppted	Do	Ppted	Do	Ppted	
Cupric acetate (acidulated)	Ppted	Imperfectly reduced	Ppted	Partially ppted	Ppted	Glucose is gradually and imperfectly oxidised
Mercuric acetate ($HgCl_2 + 2Na\overline{A}$)	Ppted	White ppte of Hg_2Cl_2 gradually formed	Ppted	Ppted	Ppted	Creatinine ppted very slowly in the cold. All others, except glucose, promptly
Fehling's alkaline cupric tartrate solution.	Red violet coloration	Immediate separation of red Cu_2O	Reduction and pptn of cuprous urate	Partial pptn and reduction	Reduction and pptn as cuprous Xanthinate	
Pavy's ammoniacal cupric solution	No change	Colour discharged	Colour discharged	Colour discharged		
Gerrard's cyanocupric solution	Do	Do	D			
Nylander's bismuth citrate solution	Do	Pptn of grey or black Bi	Pptn of Bi	No change		
Knapp's mercuric cyanide solution		Pptn of grey Hg				
Sacchse's (Nessler's) mercuric iodide solution		Do				
Braun's alkaline picric acid solution		Brown-red colour	No change	Blood red coloration		
Wender's alkaline methylene blue solution		Colour discharged	Do	Colour discharged		
Crismer's alkaline safranine solution		Do	Do	No change		

When very small quantities of sugar are to be sought for, the removal of the interfering constituents of urine is absolutely necessary, and the neglect of this preliminary treatment has given rise to many erroneous observations on the presence of sugar

Urine may be conveniently clarified, and freed from albumin (if present), uric acid, phosphates, sulphates, and colouring matters, by precipitation with neutral lead acetate   One-fourth of its measure of a 10 per cent. solution of lead_acetate in water is sufficient to throw down all the precipitable matters from almost any urine   The precipitate is bulky, but filters readily, especially if the urine be first heated to boiling and the hot reagent added to the boiling liquid   This mode of operating avoids the risk of loss from bumping which is apt to occur when the liquid containing the dense lead precipitate is heated [1]   Addition of basic lead acetate to the filtrate from the precipitate produced by the neutral salt removes certain bodies which escaped precipitation previously, but there is no material advantage in its use   In either case, the filtrate from the lead treatment is colourless, or nearly so, and is well fitted for optical examination or for testing by the phenyl-hydrazine reaction [2]

Mercuric chloride is a very perfect precipitant for urinary constituents   When sodium acetate is also

[1] Three per cent of lead acetate in fine powder may be substituted for the solution prescribed in the text   The powder should be added to the boiling hot urine, which should then be well agitated and filtered   This plan avoids dilution and the consequent correction for the volume of reagent added

[2] According to some observers, uric acid is incompletely precipitated by treating the urine with neutral lead acetate only, addition of basic acetate being requisite for its complete removal from solution   This is opposed to the author's experience, who has specially examined the filtrate from the precipitate by neutral lead acetate by the murexide test with negative results   It has been fully established that no sugar is carried down from solution by either neutral or basic lead acetate, but if ammonia be subsequently added glucose is completely removed as a compound with lead hydroxide, which can be washed without decomposition   This reaction is employed by Brucke and by Pavy for the isolation of sugar from urine

added, as recommended by G Stillingfleet Johnson, mercuric acetate is potentially present, and this is even a more perfect precipitant than the chloride Johnson recommends that the urine should be treated with 5 per cent of its volume of a cold saturated solution of sodium acetate, and 25 per cent of a cold saturated mercuric chloride solution [1] This treatment occasions an immediate voluminous precipitate containing albumin (if present), uric acid, xanthine, phosphates, colouring matters, &c Creatinine is not precipitated immediately in the cold, but separates completely in the course of a few days, even at the ordinary temperature If the liquid be boiled for a few minutes the creatinine is completely precipitated, and this behaviour affords by far the best means of separating it from urine The urea is partially, but not completely, precipitated on boiling Glucose is gradually oxidised at a boiling heat, but the reaction does not materially affect the detection of traces in the filtrate The separation of the excess of mercury from the filtrate from the mercurial precipitate presents some difficulty Sulphuretted hydrogen is not available for the purpose, as a fixed reducing body is stated by Johnson to be formed by its action, which body interferes with the subsequent detection of sugar G S Johnson recommends the careful addition of ammonia, with subsequent filtration from the resultant precipitate It is essential that the precipitation of the mercury should be perfect, as any remaining in the liquid will be reduced to metal by the glucose when the liquid is subsequently treated with caustic alkali and boiled, as it necessarily is whether Pavy's ammoniacal cupric solution or picric acid be employed for determining the glucose The a u t h o r has found

[1] The proportion of sodium acetate used by Johnson is considerably in excess of that requisite to react with the mercuric chloride

ammonia uncertain in its action, and prefers to boil the liquid for five minutes or so with zinc-dust[1] This precipitates the mercury perfectly, and the zinc, which passes into solution in its place, does not interfere with the subsequent application of the phenylhydrazine or picric acid tests, or the determination of the glucose by Pavy's solution   When the latter method is to be used, it is convenient to treat the filtrate from the precipitate produced by zinc with an equal measure of strong ammonia, which will ensure the zinc remaining in solution, and will furnish a constant supply of ammonia during the titration

As pointed out by the a u t h o r, in cases where the mere detection of glucose is sufficient, there is no occasion to remove the mercury from the solution An excess of potassium iodide may be added, which will prevent precipitation on subsequently adding caustic alkali, and if the liquid be then boiled a grey precipitate of mercury will be produced if sugar be present   Unfortunately, this reaction cannot readily be made quantitative

′Reaction of Glucose with Copper Solutions.

The reduction of cupric compounds to cuprous by reaction in alkaline solution with glucose is one of the best known and deservedly popular methods of testing for sugar in urine.   There are, however, several serious sources of error connected with the reaction, and ignorance or neglect of these has not unfrequently led to mistaken diagnosis and consequent improper medical treatment

Solutions of the ordinary blue and green salts of copper on treatment with an alkali yield a pale blue precipitate of c u p r i c   h y d r o x i d e   or   hydrated

[1] Dr James Edmunds recommends that the cold liquid should be poured through a column of zinc dust contained in a Mohr's burette (one third filled) and retained in position by a plug of glass or cotton wool

oxide of copper, having the composition CuO_2H_2, or CuO,H_2O When the liquid containing this precipitate is boiled, the hydroxide loses its water of constitution and is converted into black c u p r i c o x i d e, CuO The cuprous salts, corresponding to cuprous oxide, Cu_2O, are mostly insoluble in water, and are readily converted into the blue cupric salts by oxidation   On addition of a caustic alkali to a cuprous salt, either in the solid state or in solution, a bright yellow c u p r o u s h y d r o x i d e, $CuOH$, or Cu_2O,H_2O, is precipitated, and this, on boiling the liquid containing it, is dehydrated and converted into red or brownish-red c u p r o u s o x i d e, Cu_2O

The precipitation of cupric oxide from cupric salts by alkalies is prevented by the presence of tartaric acid, citric acid, glycerin, the sugars, and various other kinds of organic matter, but these substances exert no similar interfering action on the precipitation of cuprous oxide from cuprous solutions   Various oxidisable bodies convert cupric salts to cuprous, by assimilating one-half of the oxygen ($2CuO = Cu_2O + O$), and this occurs with special facility at the boiling point and in the presence of free caustic alkali   It follows, therefore, that if caustic potash or soda be added to a solution of cupric sulphate to which sufficient of an interfering agent has been added, no precipitate will be formed, the copper remaining in solution and communicating a deep blue colour to the liquid   If a body capable of abstracting oxygen from cupric oxide be now added, reduction to the cuprous state will ensue, and the yellow cuprous hydroxide or red cuprous oxide (according to the temperature of the liquid and other conditions of the experiment) will separate   With phenyl-hydrazine, reduction and consequent precipitation occurs immediately, and at the ordinary temperature   With glucose, it occurs slowly

in cold solutions, but immediately on boiling, while cane-sugar does not reduce the copper solution even at a boiling heat, although it shares with glucose the property of preventing the precipitation of cupric solutions by alkalies

Upon the foregoing facts the various modifications of the copper test for glucose have been based

TROMMER'S TEST —If urine or other liquid containing glucose be treated with sufficient of a solution of copper sulphate to render the liquid greenish, and a solution of caustic potash or soda be then added, a deep blue liquid will be formed if sugar in notable amount be present   On allowing the liquid to stand for some hours in the cold, a yellow precipitate of cuprous hydroxide or red precipitate of cuprous oxide will be produced, and the same precipitate will be obtained immediately on boiling the liquid Trommer's modification of the copper test is merely of qualitative value, and should be resorted to only in cases where the conditions make the application of Fehling's form of the test impossible

FEHLING'S TEST —In Fehling's form of the copper test for glucose, reliance is not placed on the sugar itself for the prevention of the precipitation of the cupric solution by an alkali, but sufficient tartrate of alkali-metal is added to form a double tartrate of copper and sodium, which is not decomposed by alkalies   One molecule of tartaric acid is able to prevent the precipitation of one molecule of copper by a caustic alkali, owing to the formation of the salt $(CHO)_2Cu(CO_2K)_2$, but it is better to employ twice the amount of tartaric acid requisite for the production of this body   Fehling's solution is therefore essentially a solution of the double tartrate of copper (cupric) and sodium or potassium, with a considerable quantity of caustic alkali   It is best prepared as follows —(a)

34·64 giammes weight of pure crystallised sulphate of copper (free from iron and moisture) is dissolved in warm distilled water, the solution filtered if necessary, and when cold diluted to 500 c c with distilled water, (b) 180 giammes of ciystallised Rochelle salt (potassium sodium tartiate) is dissolved in about 300 c c of hot watei, the liquid filtered if necessary, and 70 grammes of good caustic soda or 100 grammes of caustic potash added to the filtiate, which is then cooled and made up to 500 c c

Fehling's Solution is prepaied by carefully adding an accurately measuied volume of the above copper sulphate solution to an equal measuie of the alkaline tartrate mixtuie The precipitate fiist formed dissolves on agitation with formation of a clear azureblue solution

Fehling's solution is affected by aii and light, undergoing a change which causes a precipitation of cupious oxide on boiling The alteration is often attributed to the formation of the isomeric modification of tartaric acid known as racemic acid, but the evidence on which this statement is made appears very insufficient It is, at any iate, an undoubted fact that Fehling's solution spoils by keeping, and hence the indication obtained with a particulai sample of the ieagent cannot be trusted unless the solution be proved to give no precipitate of cuprous oxide when diluted with an equal measuie of water and boiled for a few minutes, The best plan is to store the cupric sulphate and alkaline taitiate solutions separately, and to mix them only when iequired for use, then proving by actual tiial that the mixture suffeis no reduction when diluted and boiled as just described

Fehling's solution is reduced by the following bodies when theii neutralised solutions are heated to boiling with it —

Carbohydrates, &c —Dextrose, lævulose, maltose, ısomaltose, lactose, galactose, xylose, aıabınose

Alcohols, aldehydes, phenols, &c —Acetaldehyde, valeraldehyde, chloıal, chloıoform, resorcınol

Acıds —Gallotannıc, glycosurıc, glycuronic, ındoxyl-sulphurıc, hıppurıc, salıcylıc, trıchloracetıc, urıc.

Bases —Creatıne, cıeatınıne, xanthıne, phenyl-hydrazıne (The last substance reduces Fehlıng's solutıon ın the cold, wıth evolutıon of nıtrogen)

Fehlıng's solutıon ıs not reduced when boiled wıth neutral or alkalıne solutıons of the followıng bodıes —

Carbohydrates, &c.—Cane-sugar, raffinose, mannıtol, dulcıtol, dextıın, arabın, cellulose

Alcohols, aldehydes, phenols, &c —Alcohol, glycerın, benzoıc aldehyde, salıcylıc aldehyde, phenol

Acıds —Acetıc, cıtııc, benzoıc, formıc, gluconıc, lactıc, lactonıc, mucıc, oxalıc, pıotocatechuıc, sulphur-ous, sulphamıdo-benzoıc ("saccharıne"), taıtaııc.

Bases —Urea

Among the products of the actıon of Fehlıng's solutıon on glucose are saıd to be the followıng bodıes —(1) Acetıc and formıc acıds (2) Certaın non-volatıle acıds, especıally taıtıonıc , an acıd form-ıng uncıystallısable salts , and an acıd whıch ıs decom-posed wıth formatıon of humus-lıke products on heatıng ıts alkalıne solutıon (3) A gummy substance (compare page 24)

Fehlıng's copper solutıon, when properly applıed, affords one of the sımplest and most popular means of detectıng glucose ın uııne The usual method of employıng ıt ıs to remove albumın, ıf present, by heat-ıng to boilıng the slıghtly acıd or acıdulated uııne, and filteııng from any precıpıtate (see page 106) The filtrate ıs then rendered dıstınctly alkalıne to lıtmus paper by a few grains of sodıum carbonate, heated to boilıng, and filtered from the pıecıpıtate of earthy

phosphates, &c   Ten c c of recently prepared Fehling's solution[1] is heated to boiling in a test-tube, some platinum wire or a few fragments of clay tobacco-pipe or pumice being added to prevent bumping   When the liquid boils, 1 c c of urine, prepared as above described, is added, and the liquid boiled again for a few seconds   If sugar be present in abundance, as in a decidedly diabetic urine, a yellowish or brick-red opacity and deposit will be produced   If a negative reaction be obtained, traces of sugar are tested for by adding 7 to 8 c.c. of the prepared urine to the hot liquid, heating again to ebullition, and then setting the tube aside for some time.   If no turbidity is produced as the liquid cools, the urine is regarded as either quite free from sugar or containing less than 0 2 per cent   If the proportion of sugar be moderate—that is, under 0 5 per cent —the precipitation of the yellow or red cuprous oxide does not take place immediately, but occurs as the liquid cools, the appearance being somewhat peculiar   The liquid first loses its transparency, and passes from a clear bluish-green to an opaque light greenish colour   This green milky appearance is said to be very characteristic of dextrose, but it would be more correct to say that its appearance indicated the presence of some substance interfering with the normal reaction of sugar   The number of these interfering bodies is very considerable, including, as it does, uric acid, hippuric acid, glycuronic and glycosuric acids, xanthine, hypoxanthine, creatinine, indoxyl-sulphates, mucus, peptones, and the milk-sugar sometimes present in the urine of nursing women   Some of these sub-

[1] It is absolutely necessary that the Fehling's solution employed for the test described in the text should remain clear when diluted with its own volume of distilled water and boiled for a few minutes   The cupric sulphate and alkaline tartrate solutions should be kept separate, and the Fehling's solution made when required by mixing them in equal volumes   Compare

stances exert a reducing action on the copper solution, while others, such as xanthine, uric acid, peptones and pigments, modify the reaction by causing the formation of a greenish precipitate instead of the separation of yellow or red cuprous oxide as normally obtained

Modified Fehling's Test —The presence of such interfering bodies render the indications of Fehling's solution uncertain and difficult to interpret when only small quantities of sugar are in question   A great improvement in the precision and delicacy of the test, as applied to urine, without in any way adding to the time required or to the complexity of the manipulation, can be effected by operating in a manner devised by the a u t h o r   This consists in a preliminary treatment of the urine with cupric sulphate or acetate, in neutral or slightly acid solution, whereby many of the constituents of urine are precipitated without in any way affecting any form of sugar

Cupric sulphate yields little or no precipitate with normal urine in the cold, but on standing or boiling a whitish or pale green precipitate is thrown down, which has a tendency to darken if the heating be continued If cupric acetate be substituted for the sulphate, or if sodium acetate be added to the cupric sulphate solution, this precipitation is much more complete, uric acid, xanthine, hypoxanthine, colouring matter, and albumin (if present) being entirely thrown down, and the phosphates and creatinine partially   The filtered liquid cannot be used for the phenyl-hydrazine test, and the presence of copper unfits it for titration by Pavy's solution, but it is admirably adapted for the detection of small quantities of glucose by Fehling's test The procedure is as follows —

From 7 to 8 c c $(= \frac{1}{4}$ oz) of the sample of urine to be tested is heated to boiling in a test-tube, and, without separating any precipitate of albumin which

may be produced, 5 c c (about 80 minims) of the solution of cupric sulphate used for preparing Fehling's test is added   This produces a precipitate containing uric acid, xanthine, hypoxanthine, phosphates, &c To render the precipitation complete, however, it is desirable to add to the liquid, when partially cooled, from 1 to 2 c c (20 to 30 minims) of a saturated solution of sodium acetate having a feebly acid reaction to litmus [1]   The liquid is next filtered   To the filtrate, which will have a bluish-green colour, 5 c c (80 minims) of the alkaline tartrate mixture used for preparing Fehling's solution is next added, and the liquid boiled for fifteen to twenty seconds   In the presence of more than 0 25 per cent of sugar, separation of cuprous oxide occurs before the boiling-point is reached , but with smaller quantities precipitation takes place during the cooling of the solution, which becomes greenish, opaque, and suddenly deposits cuprous oxide as a fine orange-yellow precipitate [2]

The foregoing description is intended to apply more especially to the examination of urine containing a comparatively small proportion of sugar, as it is in such cases that the interference of the co-occurring substances is most marked and objectionable   But the method is equally applicable to the examination of strongly diabetic urine   In such cases, however, the

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[1] Sufficient acetic acid should be added to the sodium acetate solution to render it feebly acid to litmus paper   A saturated solution of sodium acetate keeps well, but weaker solutions are apt to become mouldy, and then possess the power of reducing Fehling's solution   Hence it is essential in all cases of importance to make a blank test by mixing equal measures of cupric sulphate solution, alkaline tartrate solution, and water, adding a little sodium acetate solution, and heating the mixture to boiling

[2] The sudden separation of the precipitate during cooling is a phenomenon difficult of explanation   Operating as prescribed in the text on urine containing but a small quantity of sugar, the precipitate is of a bright orange yellow colour , but when typical diabetic urine is under treatment, or a solution of pure sugar is employed, the cuprous oxide is thrown down as a bright brick-red or brown precipitate   The modified colour of the precipitate yielded by slightly saccharine urine appears to be due to the extractive matters present

measure of urine taken should be reduced from 7 or 8 to 1 or 2 c c (= 15 to 30 minims), or even less, water being added to bring the volume up to 7 or 8 c c

It is evident that in this modification of the ordinary Fehling's test, advantage is taken of the very general precipitating power of cupric acetate to remove from the urine the great majority of those substances which interfere with the detection of diabetic sugar , and which by themselves either reduce the alkaline copper solution, retaining the cuprous oxide in solution, or produce a flocculent precipitate which masks the true reaction of sugar.   Operating as above described, no greenish turbidity refusing to settle is produced, and hence the separation of any cuprous oxide is very readily observed

In employing the foregoing modification of the copper test, it must not be forgotten that a solution of cupric acetate, even when acidulated with acetic acid, yields a precipitate of cuprous oxide when boiled with a solution of glucose   This reaction, first observed by B a r f o e d, and confirmed by M u l l e r and the a u t h o r, is liable to vitiate the test for small quantities of sugar, unless the prescribed method of working is strictly followed   Hence it is clearly important that the sodium acetate should not be added to the boiling hot solution, or the liquid boiled after its addition, or there will be danger of partial reduction by the sugar before adding the alkaline tartrate   But if the sodium acetate solution have a distinctly acid reaction, and its addition to the liquid be not made till the latter has somewhat cooled, the author has fully satisfied himself that there is no possibility of error from this cause.

A further practical advantage attending the employment of the foregoing modification of Fehling's test, is the complete precipitation of any albuminous

matters by the cupric sulphate solution without the necessity for an additional filtration , and the removal of these bodies at a prior stage prevents the formation of ammonia, which unavoidably ensues to a greater or less extent on boiling their solutions with caustic alkali The formation of ammonia in this manner interferes with the detection of minute quantities of sugar by Fehling's test, since the cuprous oxide produced is held in permanent (colourless) solution

The presence of any considerable quantity of ammonia, whether due to fermentation of the urea or other cause, absolutely invalidates the indications of Fehling's test, except, of course, when using Pavy's modification of it (page 66), in which a strongly ammoniacal solution of the copper reagent is intentionally employed

On adding Fehling's solution to a solution of creatinine, a green liquid is produced, and on boiling this a yellow coloration is observed, without, however, any separation of cuprous oxide In the case of urines rich in creatinine, this behaviour causes interference with the detection of glucose, the combination of the yellow and blue colours resulting in a green, and in addition the creatinine compound is said to have the power of preventing the precipitation of cuprous oxide by glucose (See also the effect of dextrin observed by Reichard and described on page 31)

In cases where the glucose present is more than equivalent to the cupric oxide in solution, as may happen when a urine containing a large proportion of sugar is being treated, the cuprous oxide precipitated often has a dark brown colour, while the supernatant liquid also has a brownish tint This behaviour is due to the caramelisation of the excess of glucose by the caustic alkali, a portion of the colouring matter produced being carried down by the precipitate

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Volumetric Determination of Glucose by Fehling's Solution —The last described modification of Fehling's test cannot be applied quantitatively, since an unknown amount of copper passes into the precipitate, while, owing to the insurmountable tendency, in many cases, of the cuprous oxide to pass through a filter, the gravimetric[1] and those modifications of the volumetric process in which filtration is necessary are not suitable for the determination of sugar in urine   In the absence of ammonia, either existing in the original urine or resulting from the decomposition of urea, fairly good results may be obtained by operating as follows —10 c c of recently prepared Fehling's solution is diluted with 40 c c of recently boiled water, and the liquid heated to boiling in a porcelain basin The urine, previously diluted with from 2 to 20 measures of recently-boiled water, is then gradually added from a burette, the liquid being kept gently boiling until the blue colour has nearly disappeared The addition of urine is then continued more cautiously, removing the source of heat and allowing the cuprous oxide to subside after each addition, when, by inclining the basin, the slightest remaining blue tint can be perceived   When the decolorisation is complete, the volume of urine which has been used is noted   This measure contains 0 050 gramme of glucose.

TITRATION OF GLUCOSE BY PAVY'S AMMONIACAL CUPRIC SOLUTION —For the examination of typical diabetic urine, containing a considerable proportion of sugar and in a perfectly fresh state, the foregoing process is fairly suitable, but it becomes troublesome

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[1] It is a fact not generally recognised, but strongly urged on the author by Dr J Edmunds, that the cuprous oxide separated in Fehling's test undergoes reoxidation with extreme facility, and to this behaviour is probably attributable the discordant results obtained by many observers who have tried to utilise the weight of the separated cuprous oxide as a means of estimating the glucose present

or inapplicable where the amount of sugar is small and the extractives correspondingly high   In such cases, and indeed for general urine testing, it may be advantageously replaced by a process devised by E W. P a v y (*Chem. News*, xl 77), who employs an ammoniacal cupric solution prepared by mixing 120 c c of ordinary Fehling's solution with 300 c c. of strong ammonia (sp gr 0 880), and with 100 c c of a 10 per cent solution of caustic soda, or the same volume of a 14 per cent solution of caustic potash [1]   The mixture is then made up with distilled water to 1 litre

The oxidising value of Pavy's solution, prepared as above, is *one-tenth* that of Fehling's solution , *i e* , 50 milligrammes (= 0 050 gramme) of glucose reduce 10 c c of Fehling's or 100 c c of Pavy's solution [2]

According to C W P u r d y,[3] the ratio of reduction of Fehling's and Pavy's solutions by glucose varies according as they are prepared with caustic potash or caustic soda   The existence of such a difference has been disproved by direct experiments made in the a u t h o r's laboratory. G B B r o o k found the oxidising action of Pavy's solution to be absolutely the same whether prepared with 56 of KHO, 40 of NaHO, or 56 of NaHO   In fact, the writer's experience is that the amount of caustic alkali and ammonia may be further varied very considerably without the oxidising value of Pavy's solution being appreciably affected

When Pavy's solution is heated with a liquid containing glucose the cuprous oxide produced does not

[1] If Fehling's solution be not at hand, Pavy's solution may be made up directly as follows —4 157 grammes of pure crystallised sulphate of copper is dissolved in about 100 c c hot water and the solution cooled , 21 6 grammes of crystallised Rochelle salt and 18 4 grammes of caustic soda (*or* 25 8 grammes caustic potash) are dissolved in hot water, cooled, and added to the copper sulphate solution   To the mixture is added 300 c c strong ammonia (sp gr 880), and the whole made up to 1 litre

[2] One molecule of glucose reduces to cuprous oxide 6 CuO existing in Fehling's solution, but only 5 CuO in Pavy's solution prepared as described in the text

[3] *Practical Uranalysis and Urinary Diagnosis,* 1894

separate as a precipitate, but remains in colourless
solution. The progress of the reaction is therefore
indicated by the gradual disappearance of the blue
colour of the liquid, and when the reaction is com-
plete—that is, when all the copper is reduced to the
cuprous state—the liquid becomes perfectly colour-
less. But ammoniacal cuprous solutions are extremely
oxidisable, and hence the blue colour indicative of
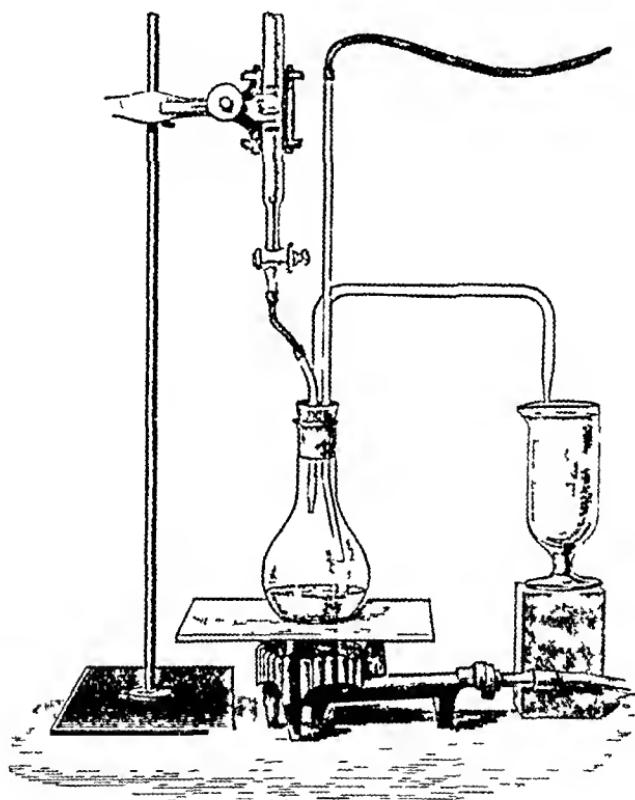
Fig. 4 —Apparatus for Pavy's Test

the existence of a cupric compound returns rapidly on
exposing the reduced liquid to the air.[1] It is necessary,
therefore, to operate in such a manner as will ensure
the complete exclusion of air during the process This
is best done by attaching the nose of the Mohr's

[1] J. E d m u n d s has pointed out, in a private communication to the author,
that the cuprous oxide precipitated in Fehling's form of the test is also highly
oxidisable, and he attributes the variable results obtained in the quantitative
application of the test to insufficient recognition of this source of error.

burette containing the urine to a tube passing through
the india-rubber stopper of a flask containing the
copper solution, as shown in figure 4. A second
tube conveys away the steam and ammoniacal gas,
while, by means of a third tube, a slow current
of coal-gas may be kept constantly passing through-
out the operation [1] The coal-gas may be dispensed
with by allowing the exit-tube to pass into an empty
Woulffe's bottle, which is connected with a flask con-
taining cold water, as suggested by A W Stokes
(*Analyst*, xii 47) If the exit-tube be provided with
a valve, the vapours may be passed direct into water
without the intervention of a Woulffe's bottle. (See
J Steiner, *Chem News*, xl 139) The author has
suggested the employment of a layer of paraffin oil to
prevent contact with the air, and the same proposal
has been subsequently made by L Peske

It is a good plan to whitewash the iron plate which
supports the flask, as by that means a marked contrast
is obtained to the blue colour of the copper solution
Stokes supports the half of an opal gas-globe behind
the flask (*Analyst*, xii 47) H. M Smith uses a
flask the lower third of which is made of white
opalescent glass [2] (*Chem News*, lxxi 165)

In carrying out Pavy's process, the author finds 25
c c of the copper solution a convenient quantity to em-
ploy This measure of the ammoniacal cupric solution
should be placed in the flask and a few fragments of
pumice or tobacco-pipe added to prevent bumping
The tubes and burette are then adjusted, a slow cur-
rent of coal-gas allowed to pass, and the contents of
the flask brought to ebullition The urine, preferably

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[1] When coal gas is employed, a brick-red film is formed on the surface of
the liquid towards the end of the titration This is not cuprous oxide, as
might be supposed, but cuprous acetylide, C_2Cu_2,H_2O, formed from the
acetylene invariably present in coal gas

[2] Obtainable from C E Muller & Co , 148 High Holborn, London, W C

freed from earthy phosphates by adding ammonia and filtering, is then gradually run in from the burette, the boiling being continued. The process is at an end when the blue colour of the liquid is wholly destroyed The end-reaction is very sharply marked, but the reduction occurs more slowly than with the ordinary Fehling's solution, and hence the process must not be hurried or too low a result will be obtained In the case of strongly diabetic urine it is desirable to dilute it to a definite volume with distilled water. On the other hand, when the proportion of sugar is small (e g , 0 2 per cent), the volume of Pavy's solution may be conveniently reduced to 10 c c When 25 c c is the volume employed, the measure of urine required to decolorise it, multiplied by four, contains 0 050 gramme of glucose

As Pavy's solution, like Fehling's original reagent, is reduced by uric acid, xanthine, creatinine, &c , as well as by glucose itself, it follows that this method also is apt to give inaccurate indications when only small quantities of urinary sugar are in question, or when an abnormally large proportion of one or more other reducing substances happen to be present A series of experiments made in the author's laboratory by G B Brook showed that the interfering substances can be most readily and perfectly removed by a plan proposed by Maly and improved by G Stillingfleet Johnson, which has been described at length on page 55 In practice, 60 c c of the urine to be tested should be treated with 15 c c of a cold saturated solution of mercuric chloride and 3 to 5 c c of a cold saturated solution of sodium acetate (see footnote on page 55) The liquid is boiled for five minutes, and filtered hot The precipitate contains all the uric acid, xanthine, creatinine, phosphates, and albumin of the urine, and a portion of the urea The preci-

pitate is washed twice with water, the filtrate acidulated with a few drops of acetic acid, boiled for ten minutes with zinc-dust,[1] and again filtered   The precipitate is washed, and the filtrate diluted to 120 c c. with strong ammonia   This liquid has half the concentration of the original urine, and is added to not more than 50 c c of boiling Pavy's solution from a burette in the usual manner   The large excess of ammonia has no prejudicial influence on the result, while it keeps the zinc in permanent solution and ensures a constant but gradual supply of ammonia during the progress of the titration   The requisite washing of the mercuric and zinc precipitates can be avoided if 50 c c of the urine be boiled with powdered mercuric chloride and sodium acetate, the liquid filtered, the filtrate treated with zinc-dust and again filtered, and a known volume of the last filtrate mixed with an equal volume of strong ammonia

Experiments made in the author's laboratory in the foregoing manner, by G B Brook, upon a limited number of samples of urine from apparently healthy persons, showed the purified liquid to have a reducing action upon Pavy's solution corresponding to the presence of from 0 05 to 0 13 gramme of glucose per 100 c c of the original urine, and yielded crystals of phenyl-glucosazone by the phenyl-hydrazine test (see page 85) Previously to the removal of the interfering substances by the mercury reagent, the same urines had a cupric oxide reducing power corresponding to from 0 1 to 0 3 gramme per 100 c c , equal to 0 44 to 1 3 grain of glucose per ounce   The difference between the amounts of apparent sugar shown before and after clarification is an indication of the proportion of interfering substances present

G Stillingfleet Johnson finds normal urine to have

1 Or by Edmunds' plan, described in footnote on page 56

absolutely no reducing action after clarification by the mercury reagent. This implies that an infinitely large quantity of the filtrate resulting from this treatment could be added to a limited volume of Pavy's solution without the blue colour of the latter being destroyed   But as the reduction of the mercuric solution with separation of calomel, and the simultaneous oxidation of the glucose, commences after a few minutes' ebullition, it is conceivable that when mere traces of sugar are present they might be entirely destroyed at this stage of the process, and that the amount found in the filtrate would be merely that which had survived the treatment.[1]

Recognising the objections attaching to any process in which glucose is subjected to the action of a hot solution containing fixed caustic alkali, A l l e i n and G a u d (*Jour Pharm Chem*, [5], xxx 305, and *Jour Chem Soc*, lxviii ii 92) have recently proposed to employ a strongly ammoniacal solution of cupric sulphate for determining sugar   The process has been tried in the a u t h o r ' s laboratory, but the reduction was found to occur so slowly that the method cannot be recommended

GLYCEROL-CUPRIC SOLUTIONS —In 1870, J L o w e suggested the use of glycerin as a substitute for alkaline tartrate in preparing an alkaline cupric solution   He gave a recipe for preparing the solution from cupric sulphate, glycerin, and caustic soda, but preferred to employ freshly precipitated cupric hydroxide instead of the sulphate (*Zeits anal Chem*, x 452)   More recently, a glycerol-cupric solution has been advocated by W. S. H a i n e s (Chicago), who prepares it by dissolving 30 grains of crystallised

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[1] Experiments made in the author's laboratory, to test this point, showed that the oxidation by mercuric acetate of glucose in very dilute solutions (0 05 to 0 10 per cent) was very imperfect, and that 80 to 90 per cent. of the glucose survived boiling with the mercuric reagent for ten minutes.

copper sulphate in 240 grain-measures of distilled water, adding ½ troy-ounce (= 240 grain-measures) of glycerin, mixing thoroughly, and adding 5 troy-ounces (5 4 English fluid ounces) of *liquor potassæ* In using this solution, it is recommended to boil about 1 drachm in a test-tube and then add two or three drops of the urine to be tested and again boil In presence of much sugar a copious yellow or yellowish-red precipitate will be thrown down. If a negative result be obtained, a further addition of urine should be made, and the boiling repeated, but it is absolutely necessary that the total volume of urine employed should not exceed six or eight drops for 1 drachm of the glycerol-cupric solution, otherwise a precipitate may be produced even in the absence of sugar

It is desirable previously to remove the earthy phosphates from the urine to be tested by adding a few drops of sodium carbonate and filtering Even with this precaution, the a u t h o r does not find the glycerin solution so delicate a test for sugar as is the ordinary tartrate solution of Fehling But the glycerin mixture has the advantage of being more readily extemporised, and is said to keep indefinitely Hence it deserves recognition as a convenient clinical test for diabetic sugar

A glycerol-cupric solution of practically the composition of that prepared by Haines' formula may be obtained by mixing 2 measures of the cupric sulphate solution employed for preparing Fehling's test with 1 measure of pure glycerin, and then adding 10 measures of *liquor potassæ*, B P

C W P u r d y (*Practical Uranalysis*, page 110) has proposed to employ Haines' glycerol-cupric solution with the addition of ammonia in place of Pavy's solution, but experiments on the process conducted in the author's laboratory showed that the method was

open to grave objections, and he is unable to recommend it

GERRARD'S CYANO-CUPRIC PROCESS OF GLUCOSE-TITRATION —A volumetric method of sugar titration, which will probably supersede all the other forms of copper determination of diabetic glucose, has been devised by A W G e r r a r d   The process, as originally published (*Year-Book Pharm*, 1892, page 400, and *Pharm Jour*, [3], xxiii 208), did not yield good results, but the w r i t e r has obtained further particulars from the inventor of the process, and has personally investigated the sources of error and the precise conditions necessary to ensure success   Gerrard's process is based on the following facts —

When a solution of potassium cyanide is added to one of a cupric salt, a double cyanide of potassium and copper is formed, according to the following equation —$CuSO_4 + 4KCy = CuCy_2,2KCy + K_2SO_4$. The double cyanide of potassium and copper thus formed is not decomposed by alkali nor precipitated by sulphuretted hydrogen. Its solution is colourless or faintly yellow   If Fehling's solution be employed instead of one of cupric sulphate, the same colourless double cyanide is formed, and the solution gives no precipitate of cuprous oxide when boiled with glucose solution.   If Fehling's solution be present in quantity more than sufficient to react with the potassium cyanide used, this extra portion will suffer reduction by glucose; but instead of the cuprous oxide being precipitated it will remain in solution, and the progress and end of the reduction will be indicated by the gradual lessening and ultimate entire disappearance of the blue colour.   Hence in outward appearance the method resembles that of Pavy (see page 67), but it has the advantage of causing no evolution of ammonia Further, the re-oxidation of the reduced solution pro-

ceeds slowly, so that, where the highest degree of accuracy is not sought, the titration, if conducted expeditiously, may be performed in an open basin, instead of in an apparatus jealously protected from ingress of air   In its improved form, as modified by G e i r a r d and the a u t h o r, the procedure is as follows —

Ten c c. of freshly-prepared Fehling's solution, or 5 c c of each of the constituent solutions, both accurately measured, should be placed in a porcelain dish, 40 c c of water then added, and the liquid heated to boiling A solution of potassium cyanide, of about 5 per cent strength, is gradually added from a burette or pipette,[1] until the deep blue colour of the liquid is nearly destroyed   The addition of the cyanide is continued, very cautiously, drop by drop, the liquid being kept constantly boiling and well stirred, until the blue colour just disappears, or only a very slight tinge of blue remains   Excess of cyanide solution must be carefully avoided, so that it is safer to discontinue its addition when the blue colour is still faintly perceptible[2]   Another 10 c c of Fehling's solution, or its equivalent, should now be added to the contents of the basin, and the urine or other sugar-holding

[1] Fletcher's graduated stopper-pipette (fig 5) is very convenient for this and similar work   It has a small slit in the upper india rubber ball which acts as a valve, and by judicious pressure the liquid in the pipette can be delivered drop by drop or rapidly as may be desired   The "*Automatic* Stopper" is manufactured by Messrs Fletchci, Fletcher, & Co , Holloway, London, N

[2] There is no occasion to go through this process every time   The double solution of potassio cupric cyanide keeps unchanged for some weeks, so that 100 c c of Fehling's solution may be diluted with about 300 c c of water, and the liquid decolorised while boiling by a 5 per cent solution of potassium cyanide   The liquid is then diluted to exactly 500 c c , and preserved in a well closed bottle   Fifty c c of this solution, corresponding to 10 c c of Fehling, are mixed with 10 c c of untreated Fehling's solution, the liquid heated to boiling, and the titration conducted as described in the text

Fig 5 —
FLETCHER'S PIPETTE

liquid dropped in *rapidly* from a burette, with constant stirring, the liquid being kept in ebullition
The end-reaction is indicated by the disappearance of the blue colour, a point which can be very sharply observed when pure solutions of sugar are being treated, but which is somewhat less distinct when urine is under examination.[1]   The volume of saccharine liquid required to decolorise the solution contains 0 050 gramme of glucose.   Hence it follows that 10 c c of urine containing 0 5 per cent of sugar will be required, and proportionately less of stronger solutions. Therefore urine suspected to contain more than 0 5 per cent of sugar should be diluted with water to a definite measure before being used   For ordinary diabetic urine, 10 measures should be diluted to 100.

Of course, if preferred, grains or minims may be substituted for the metric weights and measures prescribed above   In such case, 100 grain-measures of Fehling's solution or its equivalent may be reduced by cyanide, and a second 100 grain-measures added. A. W Gerrard has patented a special form of burette for use with the process[2]   This is so graduated as to allow the percentage of sugar contained in the urine to be read off at once without calculation

For simplicity, accuracy, and general convenience, Gerrard's cyano-cupric process is preferred by the w r i t e r to any other method of titration based on the reducing power of glucose   No doubt the other reducing constituents of urine (*e g* , creatinine, uric acid, &c) exercise the same disturbing influence on the results as in other methods, but they do not visibly interfere with the indications

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[1] Only the second measure of Fehling's solution suffers reduction   Experiments in the writer's laboratory, by Arnold R Tankard, have proved that the reducing power of invert sugar solution on the cyano-cupric reagent is securately as stated in the text

[2] Obtainable from Messrs Gibbs, Cuxson, & Co , Wednesbury

Reaction of Glucose with Bismuth Compounds.

A test for glucose which has the merits of simplicity and convenience, is the bismuth test due to B o t t g e r. The urine to be examined is mixed with an equal measure of *caustic* potash or soda solution, and a small quantity (as much as can be taken up on the point of a pen-knife) of basic nitrate of bismuth added On boiling the liquid, metallic bismuth will separate as a grey deposit or black metallic mirror if sugar be present in notable amount. N y l a n d e r has improved this test by substituting a solution of sodio-tartrate of bismuth for the solid oxy-nitrate employed by Bottger 2 5 grammes of the basic nitrate of bismuth (free from silver) and 4 grammes of Rochelle salt are dissolved in 100 c c of an 8 per cent solution of caustic soda On boiling 10 c c. of this reagent, filtered if necessary, with 1 c c of urine for a short time, the liquid will turn black if notable traces of glucose be present Albumin, blood, and other sulphuretted bodies are liable to cause confusion by precipitating black bismuthous sulphide, and hence must be removed before the test is applied [1]

A J o l l e s (*Chem Centr*, 1890, ii 609) states that with Bottger's test 0 08 per cent of sugar can be detected in urine, and this limit is reduced to 0 01 per cent if the urine be previously boiled with a solution of sodium chloride At least 50 c c of the urine should be employed to 5 c c of Nylander's reagent, and the liquid should not be boiled more than two minutes The presence of such substances as rhubarb, turpentine, kairine, quinine, sulphur, arsenic, mercury, iodides, and salicylic acid should be avoided A brown colour is produced in presence of considerable

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[1] Experiments in the author's laboratory showed that 1 c c of a 0 1 per cent solution of glucose, when boiled with 10 c c of Nylander's reagent, gave a grey coloration, and a light grey mirror appeared on the tube With 1 c c of a 1 0 per cent glucose solution, the liquid became greenish-black, and a metallic mirror was formed on the tube

quantities of uric acid, but the depth of tint from this cause does not increase beyond a certain point, whereas that due to sugar continually darkens

Reaction of Glucose with Mercuric Compounds.

Several methods of determining glucose have been based on its reaction with mercuric compounds   Thus an alkaline solution of potassio-mercuric cyanide has been recommended by K n a p p , an alkaline solution of potassium mercuric iodide (" Nessler's reagent ") by S a c h s s e , and a solution of mercuric acetate by H a g e r.   The mercury of the first two solutions is, on boiling, promptly reduced to the metallic state, and that of Hager's reagent more gradually to the mercurous condition   Hence in presence of chloride, as when the mercuric acetate is extemporised by mixing solutions of mercuric chloride and sodium acetate, a precipitate of mercurous chloride is formed on boiling the liquid for a short time (compare page 72)

The objections to the mercurial solutions are much the same as apply to those of copper, and they present no advantages to warrant an addition to the already numerous methods of determining diabetic sugar

Reaction of Glucose with Organic Colouring Matters.

PICRIC ACID TEST FOR SUGAR —Among the many coal-tar dyes and other organic compounds which suffer reduction in alkaline solution by glucose is the substance commercially known as " picric acid "   This body has the constitution of a t i n i t r o - p h e n o l, and is obtained by the action of excess of strong nitric acid on carbolic acid —

$$C_6H_5 OH + 3HNO_3 = C_6H_2(NO_2)_3 OH + 3H_2O$$
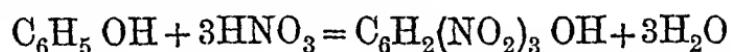
Picric acid, which occurs in pale yellow crystals, sparingly soluble in cold water, but more readily in

alcohol, ether, and benzene, readily suffers reduction in hot alkaline solution to p i c r a m i c a c i d, a body having the constitution of triamido-phenol —

$$C_6H_2(NO_2)_3\,OH + 9H_2 = C_6H_2(NH_2)_3\,OH + 6H_2O.$$
Picric Acid   Picramic Acid
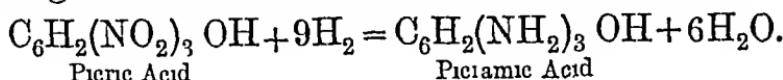
In practice, it is convenient to employ a cold saturated aqueous solution of picric acid, mixed with an equal measure of normal caustic soda or potash solution (or *Liquor potassæ*, B P)[1] The liquid is then boiled, and the urine or other liquid to be tested for glucose added, the boiling being repeated for a minute or so If glucose be present, the solution will darken from the yellow-orange colour characteristic of a hot alkaline solution of picric acid to an intense brownish-red colour, so deep as to be opaque in presence of more than traces of sugar[1]

This reaction, originally proposed as a test for sugar by C D B r a u n (1865), has been strongly advocated of late years by Sir G J o h n s o n, who has described a colorimetric application of it by which estimations of diabetic sugar may be obtained[2]   Picric acid is

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[1] The picric acid can conveniently be mixed with the alkali before it is required for use   If caustic potash be employed, an immediate deposition of beautiful crystals of the sparingly soluble potassium picrate is produced, a mere fraction of the picric acid remaining in solution   If caustic soda be substituted, a similar effect is produced in cold weather, but the separation of the sodium picrate is by no means so prompt or complete as that of the potassium salt   Indeed, a permanent and serviceable reagent can be prepared by mixing two measures of cold picric acid with one of normal soda solution, disregarding any separation of crystals which may occur

In either case it must be remembered that alkaline solutions of picric acid are much deeper in colour than those of free picric acid, and that on heating the colour becomes intensified   Impure picric acid gives a still deeper colour

[2] Sir George Johnson compares the colour yielded by the sample of urine in question with a permanent standard solution prepared as follows —

> ℞ —Liq Ferri Perchlor Fort (B P , sp gr 1 42),   ʒj
> Acid Acet Glacialis (B P , sp gr 1 058),   ʒiv
> Liq Ammoniæ (B P , sp gr 0 959),   ʒvj
> Aquam Destill ,   ad ʒiv

Mix the iron solution with the acid, then add the ammonia, and dilute with water up to 4 fluid ounces   This solution is of the same depth of colour as that produced by a solution of glucose containing 1 gram per fluid ounce, when

not affected by uric acid, but unfortunately creatinine gives with it a reaction very similar to that yielded by glucose. As a consequence, normal urine yields a blood-red colour when treated in the cold with an alkaline solution of picric acid, and the colour becomes more intense on boiling This behaviour renders the positive detection of co-occurring traces of sugar very difficult. In fact, for use with untreated

boiled with picric acid and alkali   It should be preserved in a perfectly tight stoppered bottle, or preferably in the tube actually employed for comparing it with the glucose solution

Sir G Johnson employs for the comparison a tube about 12 inches long and 1 inch in diameter, graduated into 100 c c , accurately stoppered and lipped   The standard solution is contained in a similar but shorter tube of exactly the same diameter, and accurately stoppered   These, under the name of "Johnson's Picro saccharometer," can be obtained from C E Muller & Co , 148 High Holborn, London, W C

In the opinion of the author, Johnson's tubes are much wider than is desirable, far better results being obtainable by the use of the narrow graduated tubes employed for estimating carbon in steel by Eggertz' colour-test, and obtainable under that description of any apparatus-maker Five c c measure of the sample of urine is treated with 5 c c of a saturated solution of picric acid and 2 5 c c of normal caustic soda or potash   The liquid is boiled for about a minute, cooled thoroughly, and made up to 20 c c with distilled water   Five c c of this liquid is poured into the graduated tube, which holds 25 or 30 c c , and is graduated to 0 1 c c   Five c c of the standard solution of ferric acetate is placed in the twin tube, and the colours of the two liquids compared by holding the tubes side by side up to a good light, and looking through them transversely   The comparison is facilitated by placing a piece of moistened filter paper between the tubes and the light

If the solution of the treated sample be lighter than the standard it may be concluded that the urine contains less than 1 grain of sugar per ounce (" All normal urines reduce picric acid to an extent equivalent to $\frac{1}{2}$ to 1 grain of glucose per fluid ounce.   This reduction is due to creatinine, and should be allowed for when the proportion of glucose present is small "—Sir G John son) If, on the other hand, the solution of the sample be darker than the standard, the former must be diluted with distilled water until they are exactly of the same tint   If the sample require dilution to more than 30 c c in order to match the tint of the standard, the process must be repeated on the suitably diluted sample of urine

The volume to which the treated urine requires to be made up, divided by five, gives the number of grains of glucose contained in each fluid ounce of the urine   Thus, if a sample of urine be diluted to four times its measure with water, and 5 c.c of this, after treatment with picric acid, require to be diluted to 23 c c in order to match the standard, then —

$$\frac{4 \times 23}{5} = 18\ 4 \text{ grains of sugar per fluid ounce}$$

This result, divided by 4 375, gives 4 26 per cent of sugar in the urine.

urine, the test is not, in the opinion of the author, so useful or trustworthy as that with safranine, where the reaction consists in the discharge of a deep red colour by sugar, instead of in its production   Further, the safranine test is not affected by the constant presence of creatinine   If the creatinine be previously removed by mercuric acetate, as recommended by G S Johnson, and the mercury subsequently entirely removed from the filtrate, as described on page 70, the picric acid test will indicate very minute quantities of glucose, and very fair colorimetric estimations may be made in the liquid thus clarified

METHYLENE-BLUE REACTION FOR GLUCOSE —The coal-tar colouring matter known in commerce as *methylene-blue* is the hydrochloride or double zinc salt of a base having the formula $C_{16}H_{18}N_3S$ OH, and the constitution of a t e t r a m e t h y l - t h i o n i n e h y d r o x i d e   By the action of reducing agents, including a boiling alkaline solution of glucose, this body is reduced to t e t r a m e t h y l - l e u c o t h i o n- i n e, $C_{16}H_{19}N_3S$, a colourless substance which undergoes oxidation with extreme facility, forming methylene-blue and other products   N W e n d e r (*Pharm Post*, xxvi 93, and *Analyst*, xviii 131) has proposed to utilise this reaction for the recognition and rough estimation of diabetic sugar   The test has been fully tried in the author's laboratory, and the following mode of employing it is substantially that prescribed by Wender —

A solution of methylene-blue is prepared by dissolving 1 part of the commercial colouring matter in 3000 parts of distilled water (= 0 333 gramme per litre) Six c c (= 90 minims) of this solution is first measured into a test-tube, and 2 c c (= 30 minims) of a normal solution of caustic potash (or *Liquor potassæ*, B P) added   The sample of urine to be tested is then

diluted to ten times its bulk with water, and 2 c c. (= 30 minims) of the diluted urine added to the alkaline solution of methylene-blue. The contents of the test-tube are then boiled for a minute or two, avoiding agitation or contact with air as much as possible, when complete discharge of the blue colour will ensue if the sample of urine contained 0 5 per cent or more of sugar. By repeating the test with 4 c c or still larger volumes of the diluted urine, smaller proportions of glucose may be approximately estimated. A good plan is to immerse in a bath of boiling water four or more test-tubes, each containing 6 c c of the methylene-blue solution and 2 of caustic alkali, to which gradually increasing measures of urine have been added, when a close indication of the amount of sugar can be obtained in the time requisite for completing a single test   Thus, if the blue colour is not discharged by 4 c c of the diluted urine, but is destroyed in the tube to which 6 c c. has been added, the proportion of sugar present is less that 0 25, but greater than 0 17 per cent

The outward similarity of the reaction of glucose with Pavy's solution to its behaviour with alkaline methylene-blue, suggested to the author the possibility of employing the latter reagent in precisely the same manner as the ammoniacal copper solution A series of experiments in this direction, made in the author's laboratory by G B. Brook, have shown, however, that methylene-blue presents several practical disadvantages. The discharge of the colour is not so prompt nor the end-reaction so distinct as in the case of Pavy's solution. The blue colour returns with the utmost facility, a reduced solution of methylene-blue being in fact one of the most delicate existing tests for free oxygen. The employment of a layer of paraffin oil is not feasible, since the free base of the

colouring matter is liberated by the alkali and dis-
solves in the oil with red colour, and, of course, is
prevented from free reaction with the glucose, while
on the other hand it is exposed to the air [1]

As a clinical test for diabetic sugar, the methylene-
blue reaction is very serviceable   The discharge of
the colour is a phenomenon far more readily observed,
and requires less judgment, than the distinction be-
tween different shades of red and brownish-red re-
quired in applying the picric acid test [2]

SAFRANINE TEST FOR GLUCOSE   Various other coal-
tar dyes are reduced and decolorised by heating with
glucose in alkaline solution   One of these, s a f r a-
n i n e, recommended by M Crismer (*Pharm Zeit* ,
xxxiii 65 , *Pharm Jour* , [3], xix 348), is especially
suitable for the purpose   Safranine occurs in com-
merce [3] as a brownish-red powder, readily soluble in
water to form a blood-red solution not liable to change
by keeping   No immediate precipitate is produced on
adding caustic alkali solution, but on standing more
or less separation occurs   On adding to the alkaline
liquid a solution of glucose, and heating it to the boil-
ing-point, the liquid is changed to a pale urine-yellow,

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[1] It is possible that the red substance dissolved by the paraffin oil is some
extraneous impurity, but the same behaviour occurs when ether is substituted,
and on separating the ethereal layer and shaking it with acid, a blue colouring
matter passes into the latter   Attempts to purify the commercial methylene
blue by precipitating the colouring matter by potassium iodide or brine did
not affect its subsequent behaviour with paraffin oil

[2] The writer found approximately 5 per cent of sugar by the methylene blue
test in a sample of diabetic urine, which, by more accurate analysis, was proved
to contain 4 9 per cent   In this case 1 part of the urine was diluted to 100
by water

[3] Commercial safranine is a mixture of several homologous bodies, of which
$C_{19}H_{17}N_4Cl$, $C_{20}H_{19}N_4Cl$, and $C_{21}H_{21}N_4Cl$ are the chief   The second of these
has the constitution of a chloride of paramidophenyl paramido phenazonium,
and the following structural formula —

$$H_2N^p\ C_6H_3 \left\{ \begin{array}{c} {}^2N^2 \\ | \\ {}_1N^1 \end{array} \right\} C_6H_4$$

$$Cl \overbrace{} {}^1C_6H_4{}^p\ NH_2$$
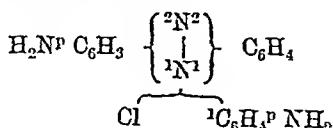
and becomes turbid from the separation of the insoluble leuco-derivative   If the glucose be not present in large excess, the red colour returns on agitating or exposing the solution to the air

Safranine is not decolorised when heated in alkaline solution with uric acid, creatine, creatinine, chloral, chloroform, hydrogen peroxide, or salts of hydroxylamine, and only slowly (but in the end completely) by solutions of albumin   Hence any discharge of colour produced by a sample of urine, preferably freed from albumin, may confidently be set down to the presence of sugar   The indifference of safranine to uric acid and creatinine has been fully verified by the a u t h o r, who employs the reagent in the following manner —Equal measures (2 c c , or 30 minims) of the urine, normal soda or potash (or *liquor potassæ*, B P), and a solution of one part of safranine in 1000 parts of water are mixed   The liquid is heated in a test-tube till it boils freely, agitation being avoided as much as possible   If the urine contain more than 0 1 per cent of sugar the liquid will be decolorised, but otherwise the red colour will remain intact or be only partially discharged   If the colour be destroyed, the test may be repeated with twice or three times the volume of safranine solution that is used of urine, every additional measure of safranine which is found to be decolorised representing roughly 0 1 per cent of sugar in the sample   If more than four or five measures are required, the urine is distinctly diabetic, and it is desirable to dilute a portion to ten times its bulk with water, and employ equal measures of this diluted urine, alkali, and safranine solution   Pellets containing a definite weight of safranine may be used

The safranine test has been fully investigated in the a u t h o r's laboratory by G B e r n a r d B r o o k It is one of the simplest and most certain clinical

tests for glucose, and deserves to come into general use for that purpose   It possesses the advantage over methylene-blue or picric acid of not being affected by uric acid or creatinine, while its application is quite as simple as that of either of these reagents   The rough quantitative indications, obtainable as above indicated, will often afford great assistance to the medical practitioner   On the other hand, attempts to base on the reaction an accurate quantitative method, by operating in the manner of Pavy, have been unsuccessful

Reaction of Glucose with Phenyl-Hydrazine.

As all the methods of detecting sugar in urine which are based on the reducing action of glucose are more or less vitiated by the presence of other reducing bodies, a special reagent for glucose has an exceptional value.   This reagent exists in phenyl-hydrazine, which the classical researches of E Fischer have shown to react with all bodies of the aldehyde and lactone classes, including glucose, with formation of well-defined compounds which are generally readily crystallisable, and insoluble, or only very sparingly soluble, in water   Thus, with a single molecule of phenyl-hydrazine, dextrose reacts to form d e x t r o s e-p h e n y l-h y d r a z i n e, $C_{12}H_{18}N_2O_5$, a body which forms fine crystals fusing at $144°$ to $145°$ C   But with excess of phenyl-hydrazine, a body of the osazone class is formed, dextrose yielding p h e n y l-d e x t r o s-a z o n e, $C_{18}H_{22}N_4O_4$ Identically the same o s a z o n e appears to be formed from lævulose, so that it may be conveniently called p h e n y l-g l u c o s a z o n e Similar compounds, differing in their melting-points, are yielded by maltose and lactose, but not by cane-sugar   The following table exhibits the distinguishing characters of such osazones as are interesting in connection with the examination of diabetic urine —

Sugar, &c	Compound (Osazone) with Phenyl hydrazine		
	Formula	Appearance	Melting-point, °C
Dextrose, Lævulose,	$\}\,C_{18}H_{22}N_4O_4$	Needles	204 to 205.
Maltose,	$C_{24}H_{32}N_4O_9$	Tables	190 to 191
Lactose,	$C_{24}H_{32}N_4O_9$	Wide prisms	200
Cane-sugar,	No compound		
Glycuronic acid,	$C_{42}H_{48}N_{10}O_{10}$	Yellow needles or brown resinous mass	115 to 115

In applying Fischer's reagent in practice, the phenyl-hydrazine is most conveniently used as the hydrochloride, a salt which forms small, thin, lustrous plates, of the formula $C_6H_5\,HN\,NH_2,HCl$ The crystals are sparingly soluble in cold water, more readily in hot The salt is almost completely precipitated from its aqueous solution by strong hydrochloric acid, which behaviour furnishes a means of readily purifying it from aniline hydrochloride and other impurities Much of the phenyl-hydrazine hydrochloride of commerce has a brownish tint, and should be purified in the manner above indicated, followed by re-crystallisation from hot water [1]

To apply the phenyl-hydrazine test, R von J a k s c h (*Zeits clin Med*, xi 20) recommends that 50 c c of the suspected urine, previously freed from albumin, should be treated with 2 grammes of sodium acetate and from 1 to 2 grammes of phenyl-hydrazine hydrochloride,[2] and the liquid heated to 100° C for

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[1] C W Purdy states that phenyl hydrazine hydrochloride must be handled with great caution, as its contact with the skin is liable to produce eczema of a malignant type

[2] Or 2 oz of urine, 40 grains of sodium acetate, and from 20 to 30 grains of phenyl hydrazine hydrochloride

half an hour. On cooling, the phenyl-glucosazone separates as an amorphous or crystalline precipitate of a yellow or brick-red colour If amorphous, the precipitate should be dissolved in hot alcohol, and the solution diluted with water and boiled to expel the greater part of the alcohol, when the glucosazone will separate at once, or on cooling, in the form of characteristic yellow needles, which melt at 205° C with decomposition Phenyl-glucosazone is nearly insoluble in cold water or alcohol, more soluble in hot water, and moderately soluble in hot alcohol. It is dissolved by glacial acetic acid to form a lævo-rotatory solution

Instead of operating in the manner prescribed by von Jaksch, the phenyl-hydrazine test may be applied in the following simple manner, which is substantially that recommended by C. Schwartz (*Pharm Zeit*, xxiii 465) —Ten c c. of the urine is heated to boiling and treated with half its volume, or a sufficiency, of a 10 per cent solution of neutral lead acetate The liquid is boiled and filtered hot Solution of caustic soda, in amount sufficient to redissolve the precipitate which first forms, is added to the filtrate, and then a little (as much as will lie on the point of a pen-knife) phenyl-hydrazine hydrochloride is dropped in The liquid is then boiled for some minutes, and then strongly acidulated with acetic acid In presence of much sugar, an immediate yellow turbidity or precipitate will be formed, but if only minute traces be present a yellow coloration is first produced, which, on cooling and standing, changes to a turbidity. In all cases, considerable time is required for the complete separation of the glucosazone, but the qualitative indication is readily and quickly obtained

Unfortunately, the phenyl-hydrazine test does not appear susceptible of being applied quantitatively,

though, of course, the intensity of the reaction and
the amount of precipitate afford a fair indication of
the proportion of sugar present. As little as 0 005
per cent ($=\frac{1}{40}$ grain per ounce) of glucose can be
detected in urine by the phenyl-hydrazine reaction

Uric acid, urea, xanthine, and creatinine in no way
simulate the reaction of glucose with phenyl-hydrazine
The only bodies which can cause confusion are gly-
curonic acid and its derivatives (page 38)   Glycuron-
ates give with Fischer's reagent a yellow, crystalline
compound resembling phenyl-glucosazone, but melting
at 150°   When the osazone is produced in an acid
solution, as in von Jaksch's modification of the test,
the glycuronic acid compound is precipitated as a
dark brown resinous mass, in which form it can cause
no confusion   According to H i r s c h l (*Zeit physiol
Chem*, xiv 377), in applying the phenyl-hydrazine
test to urine, the liquid should be heated on the
water-bath for at least an hour   The fine, bright
yellow needles of phenyl-glucosazone, melting at
204° C , are readily distinguished from the brownish
amorphous compound, melting at 150°, produced by
glycuronic acid , but if the heating be not continued
for the prescribed time, the latter compound may be
deposited in a crystalline state, and be mistaken for
glucosazone   Hirschl believes that traces of glycuronic
acid compounds are normally present in human urine

In all doubtful cases the indications furnished by
the production of a turbidity or precipitate with
phenyl-hydrazine should be confirmed by obtaining
the osazone in a crystalline form, and examining it
under the microscope   If possible, the melting-point
should be observed, and in making this determination
it is essential that the heating should be conducted as
rapidly as possible, as the compound readily undergoes
decomposition at a temperature approaching its melt-

ing-point   The coloration obtained on moistening a crystal of the glucosazone with a drop of concentrated sulphuric acid is also an important means of identification   Glucosazone acquires an olive-green colour, and on subsequently adding a minute quantity of manganese dioxide a fine purple colour is obtained, closely resembling that yielded by strychnine under similar treatment, but not undergoing the subsequent change to cherry-red which characterises the alkaloid (A H Allen)

The author has observed that phenyl-glucosazone is readily dissolved by ether from its acidulated aqueous solutions   On separating and evaporating the ether, the glucosazone can be dissolved in alcohol, and crystallised by adding water and evaporating, as already described

According to Roos, human urine always gives an affirmative reaction with the phenyl-hydrazine test, and the same is said to be true of dogs' urine, while the excretion of rabbits gives especially well-formed crystals   By the application of the phenyl-hydrazine test to a limited number of samples of urine from persons apparently in perfect health, the author satisfied himself of the presence of minute traces of sugar in each case

According to von Jaksch, no sugar can be detected by the phenyl-hydrazine test in the urine of persons poisoned by arsenic, potash, or sulphuric acid, but it always indicates the presence of sugar in the urine of those poisoned by carbonic oxide or other irrespirable gases   It is not improbable that the inhalation of the products of the imperfect combustion of coal-gas, as evolved from the faultily constructed gas-stoves now so commonly used, may account for some of the cases in which traces of sugar have been found in the urine of persons apparently in perfect health

Reaction of Glucose with Benzoyl Chloride.

If a solution of glucose be shaken with a little b e n z o y l   c h l o r i d e, C_6H_5COCl, white crystals of a compound of the two bodies gradually separate. W e d i n s k i has employed the reaction to demonstrate the existence of traces of glucose in normal human urine; but the behaviour of co-occurring substances must be studied more fully than has hitherto been done before the indications of the test can be fully accepted

ACETONURIA.

The later stages of diabetes are generally characterised by the appearance in the urine of certain substances which are evidently products of incomplete oxidation   Among these are h y d r o x y b u t y r i c   a c i d,   a c e t o n e,   and   a c e t o - a c e t i c   e t h e r   These bodies probably have their origin in the proteids of the food, for their amount is independent of any addition of carbohydrates to the diet but becomes greater with increased proteid metabolism   They do not occur in all cases of diabetes, but generally in the more severe forms of the disease, in which the destructive metabolism of proteids is augmented   In the last stage of diabetes, when coma sets in, the amount of hydroxybutyric acid increases, while that of acetone diminishes.

The occurrence of hydroxybutyric acid, aceto-acetic ether, and acetone is not confined to diabetes, but has been observed in many other maladies, including fever, however caused; cancer, under certain conditions, and in acetonæmia, apparently produced by ingestion of alcohol   Hence the formation of acetone and the allied anomalous products of metabolism may not improbably be a consequence of certain complications which accompany diabetes, and not of the disease

itself   The occurrence of aceto-acetic acid or its ethyl ester has been noticed in the urine of healthy persons after prolonged fasting, and minute traces of acetone exist in normal urine   The usual proportion of acetone excreted is stated to be 0 010 gramme per diem, but this is said to be increased to 0 500 gramme per diem, or even more, in marked cases of acetonuria

The relationship existing between acetic acid, aldehyde, acetone, aceto-acetic acid, and hydroxybutyric acid is shown by the following formulæ —

Acetic acid, .	$CH_3—CO—OH$
Aldehyde, . .	$CH_3—CO—H$
Acetone (dimethylketone),	$CH_3—CO—CH_3$
Aceto-acetic acid (diacetic acid),	$CH_3—CO—CH_2—CO\ OH$
Hydroxybutyric acid,	$CH_3—CH(OH)—CH_2—CO\ OH$

Acetone.   Dimethyl-ketone $CH_3\ CO\ CH_3$

Acetone results from the dry distillation of acetates, especially those of barium and calcium, and may be obtained in various other ways   It is a colourless, limpid liquid of peculiar ethereal odour and burning taste   It is miscible in all proportions with water, alcohol, and ether, but is insoluble in a *saturated* solution of calcium chloride

Acetone has many of the characters of the aldehydes, and like them unites with the acid sulphites of the alkali - metals to form crystalline compounds   On adding to a tolerably strong solution of acetone a cold saturated solution of bisulphite of sodium, the soda compound separates out in shining scales

When treated in solution with diazobenzene-sulphonic acid and a little sodium amalgam, acetone yields a dark red coloration   Aceto - acetic ether behaves similarly, while aldehyde gives a violet colour

B a e y e r and D r e w s e n have proposed a test for acetone based on the formation of indigo-blue   A few crystals of the highly explosive ortho-nitro-benzalde-

hyde are dissolved by the aid of heat in the suspected urine On cooling, the reagent separates as a white cloud The liquid is now made alkaline with dilute caustic alkali, when a yellow colour, changing in a few minutes to green and indigo-blue, will be developed if acetone be present

The test is more satisfactory and delicate if applied to the first portion of the distillate from the suspected urine To this, a solution of the nitro-benzaldehyde should be added, and the liquid made alkaline If the change of colour from yellow to green and blue occurs tardily, the liquid may be shaken with chloroform, which dissolves the nascent colouring matter

Acetone reduces Fehling's solution If the test be applied to a distillate from the urine, of course all interference from glucose and other fixed reducing agents will be avoided

P Chautard (*Bull Soc Chem*, xlv 83) recommends for the detection of acetone that 200 c c of urine should be distilled at a gentle heat, and the first 15 c c of the distillate treated with a solution of rosaniline acetate previously decolorised with sulphurous acid [1] If acetone be present, a fine reddish-violet coloration is produced, the intensity of which varies with the amount It is stated that less than 0 01 per cent of acetone can be detected in urine in this manner

Villiers and Fayolle (*Bull Soc Chem*, 1894, page 693, and *Jour Chem Soc*, lxvi 2, page 489) state that the crimson reaction with the rosaniline reagent

[1] The reagent is prepared by mixing 30 c c of a solution of magenta (rosaniline hydrochloride) containing 1 gramme per litre, 20 c c of a solution of sodium bisulphite of 1 31 sp gr, 3 c c of strong sulphuric acid and 200 c c of water Villiers and Fayolle prepare their reagent by adding to a solution of magenta such an amount of sulphurous acid as will just decolorise it after long standing in a closely stoppered bottle This reagent is very sensitive to all oxidising agents It gives a red colour even on prolonged exposure to air Glucose, galactose, and invert sugar give a coloration as intense as aldehyde, but lævulose, cane sugar, maltose, and lactose give no reaction until the lapse of some days

is not produced by pure acetone, but is due to aldehyde, which they state is almost always formed simultaneously with acetone   It seems probable that the reaction is common to both bodies, but is capable of detecting a much smaller amount of aldehyde than of acetone, since M o h l e r found that 0 01 gramme per litre of aldehyde but not less than 0 5 gramme of acetone could be detected in spirits by this test

For the detection of acetone in urine, L e g a l (*Jour Pharm* , [5], xviii 206) adds some drops of a concentrated solution of sodium nitroprusside, and makes the liquid slightly alkaline with caustic potash When the red coloration first produced has disappeared, a little acetic acid is added, which in presence of acetone will produce a deep violet coloration

A somewhat modified form of the above test has been described by L e N o b e l (*Chem Centralb* , 1884, page 626)   He adds to the urine a solution of sodium nitroprusside, previously made alkaline by caustic soda and so dilute as to have only a slight red tint   If acetone be present, even to the small extent of 0 0002 gramme, a ruby-red colour will be produced, changing in a few moments to yellow, and on acidulating the liquid and boiling, a greenish-blue or violet colour is developed [1]

The most delicate test for acetone is that based on its ready conversion into iodoform when treated with caustic alkali and a solution of iodine   The following equation represents the final reaction, but the highly unstable potassium hypoiodite is probably formed as a first product

$$C_3H_6O + 4KOH + 3I_2 = 3KI + KC_2H_3O_2 + CHI_3 + 3H_2O$$
Acetone   Acetate   Iodoform
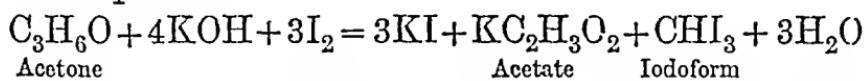
To apply the iodoform test, 10 c c of the suspected

[1] The nitroprusside test has been applied very successfully by A A s h b y to the detection of acetone in methylated spirit and liquids containing it The orange yellow colour given by aldehyde can scarcely be mistaken for the far more intense colour produced by acetone

liquid should be treated with five or six drops of a 10 per cent solution of caustic potash or soda, and the liquid warmed to about 50° C A solution of potassium iodide, fully saturated with free iodine, is next added drop by drop, with agitation, until the liquid becomes permanently yellowish-brown, when it is carefully decolourised by a further cautious addition of the caustic alkali solution Any iodoform will now be apparent as a yellowish turbidity, gradually depositing at the bottom of the liquid as a yellow dust, which, when examined with a lens, is seen to be crystalline Under a power of 300 diameters, its appearance is very characteristic, the usual forms being hexagonal plates, stars, and rosettes The formation of iodoform is pre-eminently a test for acetone, but it is not peculiar to that substance, being also produced by alcohol and its homologues, aldehyde, various ethers, turpentine, sugar, &c On the other hand, it is not given by pure methyl or amyl alcohol, chloroform, chloral, ether, or glycerin, by acetic, formic, or oxalic acid, nor by acetal till after the addition of acid With acetone the reaction occurs immediately and in highly dilute solutions, whereas alcohol and most of the other above-named bodies are converted into iodoform only slowly and in concentrated solutions

For the quantitative determination of acetone in urine by the iodoform reaction, H Huppert (*Zeits anal Chem*, xxix 632) recommends a method devised by Messinger (*Berichte*, xxi 3366) To get rid of interfering bodies, such as phenol, ammonia, and nitrous acid, 100 c c of the urine should be previously distilled with 3 c c of acetic acid, the distillate redistilled with 1 c c of dilute sulphuric acid, and if nitrous acid be present a third distillation with some urea is directed

Messinger's method for the estimation of acetone, as modified by Collischonn (*Zeits anal Chem*, xxix 562), is as follows —Twenty c c of normal caustic soda, free from nitrites, is added to the distillate obtained as above, and 30 c c of $\frac{1}{5}$ normal iodine solution added with constant agitation   The shaking is continued for four or five minutes, while the liquid is warmed to a temperature not exceeding 60° C   The liquid is then acidified with dilute hydrochloric acid (sp gr 1 025), an excess of decinormal sodium thiosulphate added, and the solution finally titrated back with the iodine solution, starch being used as an indicator

Every 762 parts of iodine which enter into combination represent 70 parts of acetone previously present

Instead of estimating the iodoform volumetrically, the excess of iodine may be got rid of by adding thiosulphate solution of unknown strength, and the iodoform extracted by agitating the liquid with ether, which is then separated, allowed to evaporate spontaneously, and the residue of iodoform weighed   142 parts of iodoform represent 70 of acetone   The result is liable to be low, owing to the volatility of iodoform even at ordinary temperatures

A Schwicker (*Chem Zeit*, xv 914) employs ammonia instead of fixed caustic alkali in making the iodoform test   Alcohol then in no way interferes, but aldehyde must be absent   The first fraction of the distillate from the suspected urine is mixed with a few drops of strong ammonia, and a few drops of decinormal solution of iodine then added   A black precipitate of nitrogen iodide is first formed, but this disappears on shaking and warming the liquid   If acetone be present in not too small a quantity, iodoform now makes its appearance, and increases on

adding a little more iodine   The characteristic odour is not greatly obscured by that of the ammonia.   If the nitrogen iodide does not readily disappear, it may be got rid of by cautiously adding a few drops of very dilute sodium thiosulphate (hyposulphite) solution. If only traces of acetone are present, more time should be allowed before removing the nitrogen iodide, and the iodine solution used should be very dilute One part of acetone in 5000 of water is said to be recognisable by this modification of the test   To-gether with iodoform, there is formed a substance which is characterised by a pungent smell and the property of exciting lachrymation   It is probably an iodine-substitution-product of acetone

E S a l k o w s k i states that sugar, when present in urine, gives rise to a substance of an aldehydic nature (probably acetaldehyde), which on distillation simu-lates many of the reactions of acetone, including the formation of iodoform

The breath and urine of persons in which acetone can be found by the iodoform and other chemical tests often have an odour of acetone   Though the occur-rence of acetone in the urine often precedes the coma which ushers in the last stage of diabetes, acetone is not itself a toxic agent   Given in large doses to men and animals, even when diabetic, it produces no coma, and it is met with in many febrile diseases in which no coma occurs

V o n J a k s c h found minute traces of acetone in the urine of healthy persons, the amount excreted being in some cases as high as 0 01 gramme ($= 0$ 15 grain) in the twenty-four hours   For its detection in such minute quantities a large quantity ($e g$, 10 gallons) should be acidulated with dilute sulphuric acid and distilled, the first fractions redistilled, and the distillate saturated with fused calcium chloride, which

unites with the alcohol while leaving the acetone unchanged The liquid is distilled on the water-bath, the distillate again treated with calcium chloride, and the process repeated until the acetone is concentrated in a comparatively small bulk of fluid, when it may be detected and determined by the methods already detailed

Aceto-Acetic Acid. Diacetic Acid Acetone-Carboxylic Acid $CH_3 CO CH_2 COOH$

This body has been obtained synthetically by treating aceto-acetic ether in the cold with a solution of an equivalent quantity of caustic potash, and after twenty-four hours acidifying the liquid and agitating with ether, which extracts the free aceto-acetic acid On evaporation, the acid is obtained as a syrupy liquid, miscible with water and decomposing at 100° C into acetone and carbon dioxide —

$$CH_3 CO CH_2 COOH = CH_3 CO CH_3 + CO_2$$
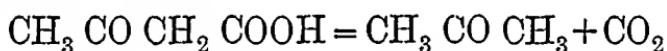
Aceto-acetic acid and its salts give a violet or claret-red coloration with ferric chloride The *barium* salt is amorphous and very soluble in water The *copper* salt is also amorphous

Ethyl Aceto-acetate, Aceto-acetic Ether, or *Diacetic Ether*, is the ethyl ester of aceto-acetic acid Its composition is $CH_3 CO CH_2 CO O(C_2H_5)$ It is prepared by the action of sodium on perfectly dry ethyl acetate Aceto-acetic ether is a liquid of an agreeable fruity odour, having a specific gravity of 1 0317 at 15° C, compared with water at the same temperature It boils at 181° C, is slightly soluble in water, and the solution gives a violet or claret coloration with ferric chloride Aceto-acetic ether resembles aldehydes and ketones in combining with the acid sulphites of the alkali-metals to form crystalline compounds, which are decomposed into their constituents by treatment with

sodium carbonate   The formation of the compound
with acid sodium sulphite may be conveniently used
for purifying the ether

Aceto-acetic ether, like acetone, gives, with diazo-
benzene-sulphonic acid and a little sodium amalgam,
a dark red coloration.

Aceto-acetic ether behaves like a monobasic acid, and
forms salts   The sodium compound is produced by the
action of sodium or sodium ethylate on aceto acetic
ether in the cold, and has the following formula —
$CH_3 CO CHNa CO O(C_2H_5)$

By the action of alkalies, aceto-acetic ether under-
goes decomposition into acetone, alcohol, and carbonic
acid   The sodium salt reacts similarly, the change in
this case being represented by the equation —

$$CH_3 CO CHNa CO O C_2H_5 + 2H_2O = CH_3 CO CH_3 + $$
$$H O C_2H_5 + NaHCO_3$$
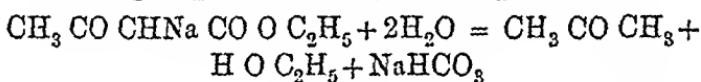
This decomposition probably occurs in the blood and
urine in cases in which acetone is present

The presence of aceto-acetic acid or its derivatives
in urine is an unfavourable symptom often preceding
the onset of diabetic coma and death   Such urine
often has an odour of acetone, and gives a violet or
claret-red coloration with ferric chloride   The colour
is destroyed by boiling, and is not produced at all if
the urine has been boiled previously.   Ferric chloride
produces a somewhat similar coloration in the urine
of persons who have taken salicylic acid, phenol,
antipyrine or thalline, but the reaction due to these
drugs is not affected by heat

By the distillation of diabetic urine, which gives the
above red or violet reaction with ferric chloride, a dis-
tillate is obtained containing acetone and alcohol
The products are usually attributed to the decom-
position of ethyl aceto-acetate, which would split
up with formation of equal molecules of alcohol and

acetone, thus — $CH_3 CO CH_2 CO O(C_2H_5) + H_2O = CH_3 CO CH_3 + CO_2 + HO(C_2H_5)$ But in a case investigated by T o l l e n s no alcohol appeared in the distillate, although much wine was taken By treating the distillate with soda and iodised potassium iodide, and weighing the iodoform produced, the acetone in the urine was found to vary from 0 093 to 0 147 per cent. From the production of the iodoform reaction by the distillate, and the absence of alcohol therein, T o l l e n s concluded that the body producing the reaction with ferric chloride was free aceto-acetic acid, and not its ethyl ester

Tollens further found that urine giving the ferric chloride reaction, when distilled slowly in presence of hydrochloric acid, yielded distillates which gave the iodoform reaction, while the residual liquid, even to the end, continued to show the ferric chloride reaction The original urine yielded to ether a mere trace of the body giving the violet reaction, but after acidification with sulphuric acid ether extracted it readily

Hydroxybutyric Acid. $CH_3CH(OH) CH_2COOH$

The kind of hydroxybutyric acid present in urine, and represented by the foregoing formula, is the β-modification It was first discovered in the urine by M i n-k o w s k i, and first obtained synthetically by acting on ethyl aceto-acetate with water and sodium-amalgam It is also formed when propylene chlorhydrin is heated to 100° with alcohol and potassium cyanide, and the nitril thus produced decomposed by caustic potash The resultant liquid is acidulated with sulphuric acid and shaken with ether The ethereal layer, when evaporated, leaves the hydroxybutyric acid as a viscid deliquescent syrup volatile in a current of steam, but converted when heated alone to 130° C , into α-crotonic acid, with loss of the elements of water As prepared

from diabetic urine, by an adaptation of the foregoing process, hydroxybutyric acid has a lævo-rotatory power of −23 4° for the sodium ray   The *sodium salt* forms very deliquescent needles   The *zinc salt* is amorphous

Hydroxybutyric acid is poisonous when introduced into the circulation   With ferric chloride it yields a claret or violet-red colour similar to that produced by aceto-acetic ether

The formation of β-hydroxybutyric acid by the action of nascent hydrogen on aceto-acetic acid has already been referred to , the quantities of the two bodies present in diabetic urine are stated by W o l p e to rise and fall together

ALBUMINOUS URINE.

NORMAL urine is almost, if not entirely, free from any trace of albumin[1] or other of the allied substances classed together under the generic name of "proteids"[2]

[1] The term "albumen" should be limited to its original signification, namely, the white of egg, the word "albumin" being applied to the most characteristic constituent thereof, and extended to other analogous substances contained in blood serum, &c. The terms albumen and albumin will then have the same relation to each other as benzol and benzene. In pronouncing the word albumin, it is correct to accentuate the penultimate, but of late years extensive custom has justified the accentuation of the first syllable.

[2] The bodies called p r o t e i d s are highly complex and, for the most part, uncrystallisable compounds of carbon, hydrogen, nitrogen, oxygen, and sulphur, having an ultimate composition ranging, according to H o p p e - S e y l e r, within the following limits —

	Carbon	Hydrogen	Oxygen	Nitrogen	Sulphur	
From	51 5	6 9	20 9	15 2	0 3	per cent
To	54 5	7 3	23 5	17 0	2 0	,,

Proteids occur in a solid viscous state or in solution in nearly all the solids and fluids of the animal economy. The different individuals of the group present differences in physical and, to some extent, in chemical properties, but all possess many chemical reactions in common, and they are united in a close genetic relationship.

W. D. H a l l i b u r t o n (*Watts' Dict. Chem.*, 1894, iv. 330) classifies proteids as follows —

I *Albumins.* Soluble in water, and not precipitated by saturating their solutions with common salt or magnesium sulphate. Coagulated by heat. Egg albumin, serum albumin, and lact albumin are the most important members of the group.

II *Globulins,* represented by serum globulin, fibrinogen, myosin, crystallin, and globin. They are insoluble in water, but soluble in dilute solutions of neutral salts, and are precipitated in an uncoagulated condition by saturating these solutions with common salt or magnesium sulphate. Globulins are coagulated by heat.

III *Albuminates*, represented by alkali albumin, acid albumin or syntonin, and metallic compounds of proteids. Acid- and alkali albumin are insoluble in water and solutions of neutral salts, but readily soluble in dilute acids and alkalies. They are not coagulated by heat.

IV *Proteoses* are proteids which are not coagulable by heat, but are mostly precipitated by certain neutral salts. They are precipitated in the cold by nitric acid, the precipitate dissolving on application of heat, and reappearing

But under particular conditions of fatigue or disease, albumin may appear in the urine

Temporary albuminuria is sometimes induced by a cold bath, especially in persons prone to kidney-disease, and it has been observed after excessive muscular exercise, as in the urine of soldiers after a prolonged march.

Any cause which leads to increased blood-pressure in the kidneys tends to induce albuminuria, and many of the cases in which it is the result of disease may be traced to this cause. Albuminuria is a constant accompaniment of the nephritis following scarlet fever, and may occur to a less marked extent in pneumonia, typhoid, and diphtheria. It may also occur in diabetes, and is then a highly unfavourable symptom

The proportion of albumin present in pathological urine varies greatly. In certain diseases the urine sometimes becomes so highly albuminous that on heating it will undergo coagulation in a manner similar to white of egg. More frequently, comparatively small amounts of albumin are found, and in the cases of convalescent patients it is often of importance to ascertain the presence or absence of mere traces of albumin [1]

as the liquid cools   Proteoses are intermediate in characters between the proteids of Groups I , II , and III and the peptones   (Compare page 120)

V *Peptones* are the ultimate products of the action of proteolytic ferments on the bodies of the preceding groups   They are very soluble in water, not precipitated by neutral salts or nitric acid, and not coagulated by heat   They give the biuret reaction, are incompletely precipitated by picric, phospho tungstic, and phospho molybdic acids, but completely by tannin, potassio mercuric iodide, and excess of absolute alcohol   Peptones are divided into *hemipeptones,* which yield leucine and tyrosine as the further result of pancreatic digestion, and *antipeptones* which do not give these products

VI *Insoluble proteids*   This class includes a number of bodies not classified above, but which resemble each other in their extreme insolubility in various reagents   Among them are fibrin, coagulated albumin and casein, lardacein, antialbumid, and gluten

[1] In cases of Bright's disease, the urine rarely contains more than 1 per cent of proteids , but taking the volume of urine at the moderate estimate of 50 oz daily, this corresponds to a loss of about 220 grains, or 14 grammes per diem   F r e u n d has pointed out that the blood of the average body contains only about 450 grammes of albumin, so that if only 7 to 8 grammes of this be lost daily the condition is alarming

	Serum Albumin	Serum Globulin	Hetero Proteose	Deutero-Proteose	Peptones	Acid Albumin	Alkali Albumin	Mucin
Dilution with water, Saturation with magnesium sulphate (page 118),	No change No change	Slight opacity Precipitate	No change Precipitate	No change No change	No change No change	No change Precipitate	No change Precipitate	No change
Saturation with ammonium sulphate (page 122),	Precipitate	Precipitate	Precipitate	Precipitate	No change	Precipitate	Precipitate	
Reaction on boiling after slight acidulation with acetic acid (page 105),	Precipitate	Precipitate	No change	No change	No change	No change	Precipitate	No change
Cold concentrated nitric acid (page 107),	Precipitate	Precipitate	Precipitate, soluble in excess or on heating, reappearing on cooling	Precipitated only on adding brine, dissolves on heating, reappears on cooling	No change	Precipitate	Precipitate	Precipitate
Metaphosphoric acid (page 113),	Precipitate	Precipitate				No change	Precipitate	
Picric acid (page 110),	Precipitate	Precipitate	Precipitate	Precipitate, soluble on heating	Precipitate, soluble on heating	Precipitate	Precipitate	No precipitate unless acetic acid be added
Potassium ferrocyanide (page 108),	Precipitate	Precipitate	Precipitate	No change	No change	Precipitate	Precipitate	
Potassio mercuric iodide (page 114),	Precipitate	Precipitate	Precipitate	Precipitate, soluble on heating	Precipitate, soluble on heating	Precipitate	Precipitate	Precipitate
Fehling's solution (biuret reaction) (page 123),	Brown red or violet	Brown red or violet	Rose pink	Rose pink	Rose pink	Brown red or violet	Brown-red or violet	Violet
Cupric sulphate,	Precipitate	Precipitate	Precipitate	No change	No change	Precipitate	Precipitate	

Detection of Albumin in Urine.

Until recently, the proteids liable to occur in urine were classed together under the general name of "albumin," but it is now recognised that several forms are of common and simultaneous occurrence, and apparently have a varied pathological significance

The table on page 103 exhibits in a convenient form the chief reactions of solutions of the proteids which are liable to occur in urine

For clinical use and medical purposes generally, it is necessary to employ simple but fairly delicate tests for the detection of albumin in urine, and many attempts have been made to fulfil the requisite conditions   The following are among those tests which experience has shown to be most generally available and reliable for the purpose, but the recognition of mere traces of albuminous matters in urine is often of great importance, and to effect this with certainty the tests must be applied with care and skill

Urine to be examined for albumin should, by preference, be the mixed excretion of the previous twenty-four hours, but it is easy to lay too much stress on this desideratum   It is better to have a carefully collected sample of the urine passed at one time than a sample of mixed urine collected under conditions open to exception. Thus, in collecting a sample of urine to be examined for albumin, it is important to reject the first ounce or two passed, in order to wash casual discharges out of the urethra The urine which follows should be passed direct into the sample-bottle, which must be scrupulously clean and may conveniently contain six ounces

Before applying any of the following tests for albumin *it is essential that the urine to be examined should be filtered*, so as to obtain an absolutely clear liquid, and to ensure its freedom from casual contami-

nation with semen, mucus, epithelial cells, or other debris from the urinary passages [1]

Previous to filtration, it is important to observe the reaction of the urine. If a slip of blue litmus-paper be promptly reddened when dipped for an instant into the urine, the liquid may at once be filtered. Urine passed during the so-called alkaline tide (page 9) may fail to redden blue litmus, or may even restore the blue colour to reddened litmus-paper. In such case, the urine, before filtration, should be acidulated by adding dilute acetic acid,[2] drop by drop, with frequent agitation to ensure perfect homogeneity, until the attainment of a proper degree of acidity is marked by the prompt reddening of a slip of immersed litmus-paper. The urine thus treated will, on filtration, yield a perfectly bright filtrate, which practically is the true urinary excretion, will give no trouble from the presence of mucin, and will yield no precipitate of earthy phosphates or other salts on boiling.

Heat Test —One of the simplest, and in many cases most satisfactory, tests for the presence of albumin in urine is that of heat. About 10 c c (or $\frac{1}{2}$ oz) of the sample, previously filtered and, if necessary, acidulated, as above described, is boiled for about one minute in a test-tube. If albumin be present, as the boiling point is approached a cloud is seen to form at the top of the liquid, and as the urine is boiled the whole of the albumin separates as a soft, white, opaque precipitate, more or less dense according to the

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[1] To ensure perfect filtration, which, when practicable, should be performed on the fresh, warm excretion, a fine close filter paper should be employed The importance of operating on carefully filtered urine has been pointed out and insisted on by James Edmunds (*Lancet*, November 9th, 1889, page 978)

[2] The use of too strong an acid should be avoided. *Dilute acetic acid* of the Pharmacopœia contains 4 27 per cent of real acetic acid, and is a suitable reagent for the purpose; or *normal acetic acid* containing 6 per cent of real acid, which can be obtained approximately by diluting 2 fluid ounces of *Acetic Acid*, B P, with 11 fluid ounces of distilled water, may be employed

proportion which may be present   On standing for
a few minutes this precipitate aggregates into distinct
flocculi, and these gradually sink to the bottom of the
test-tube, leaving the supernatant liquid clear   After
standing for twenty-four hours, the volume occupied
by the precipitate will afford a rough indication of the
proportion of albumin present   (Compare Esbach's
test, page 111)   In highly albuminous urines the
precipitate is occasionally so voluminous as to cause
the coagulation of the entire liquid

By the foregoing simple mode of procedure, any
proportion of albumin greater than traces will be
readily detected   For the detection of smaller
quantities, equal measures of the carefully filtered
acidulous urine should be placed in two exactly
similar test-tubes   The liquid in one tube is then
boiled, when, on comparing its appearance with that
in the other tube, placed side by side with it, the
faintest opalescence will be readily perceived, especially
if the tubes be observed in a proper light, with a
black background for the line of vision   An alterna-
tive plan is to boil the upper part of a column of urine
in a somewhat long test-tube by means of a small
flame   Any albumin in the upper heated portion of
the liquid will thus be coagulated, and will present
a marked contrast to the pellucid lower layer

If not in a distinctly acidulous condition, human
urine, on boiling, often yields a precipitate of earthy
phosphates, while calcium carbonate is sometimes
thrown down from the urine of herbivorous animals
These precipitates are readily and completely re-dis-
solved on adding a few drops of acetic acid, with
agitation between each addition, while a precipitate
of albumin will remain unchanged under such treat-
ment   A pulverulent precipitate of earthy phosphates,
usually $CaHPO_4$, is easily distinguished from the fine

flocculi into which an albuminous precipitate soon aggregates   From alkaline urine, albumin is not thrown down by boiling

In conducting the heat test for albumin, one of the essentials of success is to have the liquid acidulated, as already stated, to a suitable extent   The urine should sharply redden blue litmus-paper, but excess of acid must be avoided   Occasionally it is desirable to make several tests on portions of the sample to which gradually increased quantities of acetic acid have been respectively added

According to T y s o n, the addition of a few drops of acetic acid may diminish an albuminous precipitate, but on adding more re-precipitation occurs   A large excess, especially if the liquid be boiled, will permanently dissolve the precipitate of albumin   Nitric acid is preferred to acetic acid by some operators, but even more care is necessary to avoid the use of an excess, and the reagent is unsuited for the study or bedside

C W P u r d y (*Practical Uranalysis*, 1894) recommends that the urine should be treated with sufficient of a saturated filtered solution of common salt to raise the gravity to about 1035   One or two drops of acetic acid should then be added and the upper portion of the liquid boiled, as already described   The addition of the brine is stated to prevent any precipitation of mucin, and hence to avoid the confusion thereby occasioned But Purdy, apparently, omits to filter the acidulous urine, which would practically remove the mucin

Nitric Acid Test —Another delicate and simple test for albumin in urine is due to H e l l e r, and is based on its coagulation by cold nitric acid   The simple addition of some of the urine to strong nitric acid contained in a test-tube, in such a manner as to prevent the liquids from mixing, suffices for the detection of notable quantities of albumin, but the

reaction is much increased in delicacy and reliability by operating in the following manner, devised by Sir William Roberts   A saturated solution of magnesium sulphate is prepared by dissolving 10 parts of the crystallised salt in 13 of hot water and filtering the liquid   To 5 measures of this solution, 1 of nitric acid of 1 42 specific gravity is added   This reagent is so dense that it is easy to avoid admixture with the urine to be tested   Some of the acid mixture is placed in a test-tube, and an equal or larger measure of the filtered urine allowed to flow gently on to the surface   carefully avoiding any mixing of the two layers [1]   In presence of much albumin a more or less opalescent zone is immediately formed at the junction of the two liquids , but when only traces are present a longer time, sometimes extending to a quarter of an hour, is requisite for the development of the band The turbidity due to albumin occurs at the bottom of the layer of urine, just above the line of demarcation, while any cloudiness due to mucin [2] always appears as a diffused haze towards the upper part of the liquid, and therefore quite distinct from the albumin ring A stratum of uric acid occasionally separates in applying this test, but it disappears on warming   With some urines a crystalline precipitate of nitrate of urea may form   but this cannot be mistaken for albumin

FERROCYANIDE TEST —Another very delicate test for albumin is to treat the suspected urine with excess of acetic acid, and then add an aqueous solution of

[1] J E Saul (*Pharm Jour* , [3], xvii 857) suggests the use of a small glass syringe instead of a test tube   About an inch of the clear urine is first drawn up into the syringe, and then a sufficiency of the reagent   In this manner the danger of inadvertently mixing the two layers is much lessened

[2] After taking copaiba balsam or sandal oil, the urine may contain resin acids, precipitable by nitric acid, and therefore liable to be mistaken for albumin In such cases, Alexander recommends that 10 c c of the urine should be treated with two or three drops of hydrochloric acid, which will precipitate the resin acids   If, on adding acetic acid, a precipitate is formed, insoluble in excess of the reagent, this consists of mucin

potassium ferrocyanide A white precipitate will form immediately, or after a short interval, if any trace of albumin be present [1] The reaction is delicate, and produced only by coagulable proteids, which is important, since peptones may be present in urine without albumin If true albumin be present, there will be paraglobulin and myosin as well This is stated to occur in amyloid degeneration of the kidney

F W Pavy employs tabloids containing citric acid and potassium ferrocyanide, instead of using solutions of the reagents [2] For clinical purposes, this plan is very convenient

G Oliver, of Harrogate, has proposed to employ strips of filter-paper impregnated with the reagents By immersing one of the potassium ferrocyanide papers and another of the citric acid papers in a little (5 c c) distilled water, the reagent can be prepared in a few minutes

C W Purdy (*Practical Uranalysis*) insists strongly on the importance of adding the acetic acid and ferrocyanide solution simultaneously, or at any rate the latter reagent before the acid In this way he states that any precipitation of mucin is wholly avoided To a test-tube half filled with the sample of

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[1] A yellow coloration is sometimes produced on adding the ferrocyanide solution to urine According to J P Karplus (*Chem Centr* , 1893, ii 496) this reaction is due to nitrites, which he states are often present in urine which has been kept more than twenty four hours, but not in the fresh excretion

[2] Pavy directs that a pellet of citric acid should be dropped into about a drachm (= 3 5 c c) of the urine, and the liquid agitated till solution occurs A ferrocyanide pellet is then added, and the fluid again agitated An immediate precipitate occurs if albumin be present If the urine be turbid from the presence of urates, it must be previously filtered or clarified by heating, but otherwise heating is unnecessary A turbidity on adding the citric acid only may be due to either uric acid or mucin In the former case, previous dilution of the sample with an equal measure of water prevents its formation , in the latter, the density of the precipitate produced after adding the ferrocyanide should be compared with that produced by citric acid alone The ferrocyanide test is less delicate when citric acid is employed than when acetic acid is used, but Pavy's modification is very useful for clinical purposes The pellets may be obtained from Mr Cooper, 66 Oxford Street, London

urine, Purdy adds "a drachm or so" of a 5 per cent
solution of potassium ferrocyanide, and after agitating
adds from 10 to 15 drops of acetic acid   No reaction
is said to be produced by mucin, peptones, urates, phos-
phates, alkaloids, or resin acids   Any precipitate is
due to albumin and nothing but albumin

Z o u c h l o s   proposes   to   substitute   *potassium
thiocyanate* (sulphocyanide) for   the   ferrocyanide.
He prepares the reagent by mixing 10 c c of a
10 per cent solution of the salt in water with 2 c c
of acetic acid

A O l l e n d o r f f (*Zeitschr anal Chem*, xxxiii
120) confirms the value of Zouchlos' test, and states
that it is capable of detecting 0 005 per cent of
albumin, while other constituents of urine, with the
exception of propeptone, have no disturbing influence

PICRIC ACID TEST —A valuable reagent for the
detection of albumin in urine is a cold saturated
aqueous solution of picric acid, first proposed by
B r a u n, and since strongly advocated by S i r G.
J o h n s o n and others   (*Brit Med. Jour*, Oct 11,
1884 , *Analyst*, ix 206) This may be added either
to the original (filtered) urine, or to a portion in which
acetic acid has failed to give a precipitate, and either
the cold or hot urine may be employed   The reagent
may either be allowed to mix with the urine or the
junction of the two layers may be observed, when a
turbidity will be produced if any trace of albumin be
present   For clinical purposes, a minute quantity of
powdered picric acid may be substituted for the
solution of the reagent   Peptones, alkaloids, pipera-
zine, and urates (when in large excess) are liable to
give precipitates with picric acid , but these pre-
cipitates are readily distinguished from that due to
albumin by their disappearance on heating the liquid
In the absence of acetic or other added acid, picric

acid does not precipitate mucin, and this fact forms a valuable distinction between that body (said by Sir G Johnson to be always present in traces even in normal urine) and true albumin

Esbach's Test —A very fair approximation to the quantity of albumin contained in urine is said to be obtainable in the manner devised by E s b a c h, who performs the test in a graduated tube, about 6 inches in length and 0 6 inch in diameter Esbach's reagent is prepared by dissolving 10 grammes of picric acid and 20 of citric acid in about 900 c c of boiling water, and after cooling making up the volume to 1 litre by adding water   (Or $\frac{1}{4}$ oz. of picric and $\frac{1}{2}$ oz of citric acid may be dissolved in 25 oz of water)   The tube is filled to the mark " U " with the urine to be tested, and the reagent added up to the mark " R "   The liquids are mixed by cautiously inverting the closed tube several times, avoiding strong agitation, and then left at rest for twenty-four hours The volume of the precipitate is then observed, each degree corresponding to 0 1 per cent of albumin[1]   The process is inapplicable to urine containing less albumin than 0 1 per cent , and samples containing more than 0 7 per cent must be previously diluted with their own or twice their measure of water, as the tubes are not graduated for more highly albuminous urines

Fig 6 — Esbach's Tube

[1] The graduation of the tubes is purely empirical   The results are commonly declared to be quite accurate enough for all clinical requirements   In the experience of the a u t h o r, the results are fairly comparable if the same conditions as to time, &c , are observed, but the absolute quantities of albumin indicated leave much to be desired in point of accuracy, and wide discrepancies occur if the reading be taken much before or after the twenty-four hours prescribed

Esbach's tubes are obtainable from E Cetti, 36 Brooke Street, Holborn London , A Gallenkamp & Co , Cross Street, Finsbury, London , Southall & Son, Birmingham , or Gibbs, Cuxson, & Co , Wednesbury

A greatly improved form of Esbach's tube has been devised by C. W. Purdy, of Chicago[1] The tube is drawn out into a blunt cone at the closed end, so that much smaller quantities of albumin can be measured. Dr. Purdy makes the further great improvement of placing the tube in a centrifugal machine (such as the Leffmann-Beam apparatus for estimating fat in milk), whereby complete separation of the albumin can be effected in a few minutes, instead of after standing twenty-four hours

TRICHLORACETIC ACID, $CCl_3 COOH$, is a reagent for albumin proposed by Raabe, and a test for which special advantages are claimed. The reagent does not precipitate peptones nor coagulate mucin. It is said to precipitate a form of albumin not indicated by either of the previously described tests, which form appears to be specially characteristic of the presence of granular epithelial, or hyaline casts. Trichloracetic acid used in saturated solution, and poured on the cold urine, will detect 1 part of albumin in 100,000 of liquid. In presence of quinine or other alkaloids a small addition produces a dense white precipitate, soluble on heating or on adding a large excess of the acid

SALICYL-SULPHONIC ACID[2] is recommended by M Roch (*Archiv des Pharm*, xxvii 998) and J A MacWilliam (*Brit Med Jour*, i, 1891, page 837) as a precipitant of all varieties of proteids in urine. In applying the test it is simply necessary to add a few crystals of the reagent to a small quantity of the clear urine and agitate, when the appearance of a turbidity or an actual precipitate will indicate the presence of albumin. The precipitate produced

[1] Obtainable from Messrs Eimer & Amend, 205 Third Avenue, New York

[2] Salicyl sulphonic acid or sulpho salicylic acid is prepared by heating salicylic acid with twice its weight of sulphuric anhydride at 100° C until dissolved. On cooling and standing brownish crystals of the compound separate. These are purified by recrystallisation from boiling water

by albumins and globulins is not affected by heat, while that due to albumoses and peptones dissolves, reappearing as the liquid cools   No normal or abnormal constituent of urine other than proteids is precipitated by the reagent, while these are very completely separated   One part of egg-albumin in 20,000 of water can be detected

METAPHOSPHORIC ACID, readily obtained by dissolving glacial phosphoric acid in cold water, is recommended by C Hindenlang (*Chem   Centralb*, 1881, page 471) for the detection of albumin in urine To obviate the inconvenience caused by the ready conversion of the meta- into ortho-phosphoric acid, L Blum (*Chem   Centralb*, 1887, page 345) recommends the following reagent —From 0 03 to 0 05 gramme of manganous chloride is dissolved in a little water, acidulated with a few c c of dilute hydrochloric acid, and treated with 100 c c of a 10 per cent solution of sodium metaphosphate (best prepared by igniting microcosmic salt in platinum) Lead dioxide is then added, in small quantities at a time, with constant agitation, the liquid is allowed to settle, and filtered   The resulting pink solution of manganic metaphosphate is used as an indicator of albumin   The reagent should be placed in a testtube and the urine to be examined filtered into it The solution is said to keep well, and to give no reaction with other constituents of urine

SPIEGLER'S TEST (*Ber*, xxv 375) consists of a solution of 8 grammes of mercuric chloride, 4 of tartaric acid, and 20 of sugar, in 200 c c of water   On running onto the surface of this reagent some of the urine (previously acidulated with a little strong acetic acid and filtered if necessary), a distinct white ring is formed at the line of demarcation if albumin be present   Globulin and hemi-albumose behave simi-

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laily, but peptones give no reaction   The addition of sugar to the reagent is simply for the purpose of increasing the density, and so avoiding mixture with the stratum of urine

POTASSIO-MERCURIC IODIDE has been recommended by Tanret as a precipitant of albumin [1]   The reagent is prepared by dissolving 1 35 gramme of mercuric chloride and 3 32 of potassium iodide in 64 c c of water, and adding 20 c c of acetic acid   Méhu states that this reagent precipitates mucin   Brasse found peptones and alkaloids to be thrown down, but the precipitates due to these bodies dissolve on heating, leaving that due to albumin   The alkaloidal precipitate is soluble in ether, the peptone precipitate insoluble   Bile-salts gave a precipitate not dissolved by heat, but distinguishable from that due to albumin by its solubility in ether

No reaction with Tanret's reagent is produced by creatine, creatinine, xanthine or hypoxanthine

Various other reagents have been proposed for the

[1] Tanret has proposed to determine the albumin volumetrically by acidulating the urine with acetic acid and adding a standard solution of potassio-mercuric iodide until a drop of the liquid yields a precipitate on addition of mercuric chloride   The method is said to give fair clinical results

F Venturini suggests a modification of Tanret's process based on the fact that mercuric chloride precipitates albumin from urine acidulated with acetic acid before reacting with potassium iodide   A standard solution of mercuric chloride, containing 10 grammes per litre, is prepared   Each 1 c c of this solution precipitates 0 0245 gramme of albumin   To 5 c c of the urine, 6 c c of a 5 per cent solution of potassium iodide is added, together with a few drops of acetic acid, and the standard mercuric chloride then added drop by drop, until a permanent yellowish red coloration is obtained   From the volume used a deduction of 1 c c is made for the excess required to show the coloration, and the difference is multiplied by the factor 0 0245 to obtain the amount of albumin

Georges (*Jour Pharm* , [5], xiii 353) utilises these facts for the detection of peptones in the following manner   The coagulable albumin is first precipitated by heating the urine   The filtrate is precipitated by Tanret's reagent, and the precipitate washed on the filter with cold water charged with acetic acid to the same extent as the urine   It is then washed with the same acidulated water heated to boiling, the washings being kept separate   The clear liquid thus obtained gives a precipitate on cooling if any trace of peptone has been dissolved

detection of albumin in urine, but their behaviour
with co-occurring bodies has either been incompletely
studied or they present no tangible advantages over
the tests already described

Grainger Stewart (*Edin Med Jour*, May
1887) is of opinion that picric acid is the most delicate
of all reagents for albumin, Tanret's reagent ranking
second   U Vetlesen, of Christiania, represents
the relative delicacy of the various tests by the
following figures —Nitric acid, 85 , trichloracetic
acid, 82 , potassium ferrocyanide and acetic acid, 82 ,
metaphosphoric acid, 72 , picric acid in solution, 36 ,
sodium sulphate and acetic acid, 25   D Campbell
Black (*Urine and Urinary Analysis*), as the result of
careful experiments, considers Tanret's reagent the most
delicate , heat, nitric acid, and the aceto-picric solu-
tion following in the order named , and ferrocyanide
being one of the least delicate of the tests tried [1]

[1] Recent experiments by E J Evans (*Pharm Jour* , [3], xxv 913), with
a view of comparing the relative delicacy of different reagents, gave the follow-
ing results with solutions of egg albumin   Solution No 1 had a strength of
1 in 40 , solution No 2 of 1 in 200 , while solution No 3 contained 1 part of
albumin in 1000 of water   Half an ounce (S c c) was employed for each
experiment   No indication is given in the paper whether dry albumin or
fresh egg albumen was employed

On boiling, No 1 showed coagulation and slight opalescence, but only
frothing was observed in the case of solutions 2 and 3

On adding a few drops of acetic acid and heating, No 1 coagulated , No
2 gave a white froth with slight coagulation , while No 3 showed a slight
froth, without any sign of coagulation or opalescence

Heated with a few drops of nitric acid, solution 1 gave a cloudy precipitate,
which became denser on heating , No 2, a white cloud in the line of the drops,
and on shaking a white opalescence , while No 3 showed only a slight froth
without opalescence

Picric acid gave with No 1 a bulky yellow precipitate, soluble in excess of
ammonia , with No 2 a yellow opalescence, and after heating and standing
for some time a yellowish precipitate , and No 3 behaved somewhat similarly

With a nitric acid solution of ammonium molybdate No 1 gave a white pre-
cipitate, separating in flocks when heated , No 2 a white opalescence , and No
3 the same

Uranium acetate produced with No 1 a yellowish-white precipitate, curdling
on heating , with No 2 a yellowish white opalescence, a precipitate separating
on heating , and much the same with No 3

Determination of Albumin in Urine.

The method of Esbach (page 111) affords quantitative results which are somewhat rough but sufficiently accurate for many purposes. More exact determinations can be obtained by precipitating the albumin, &c., by a suitable reagent, and drying and weighing the washed precipitate, or estimating the nitrogen contained in it[1]. From the nitrogen found, the proteids are calculated by multiplying by the factor 6 3[2]. Some observers employ the factor 6 25, and others as high a factor as 6 37. 6 3 is a fair average figure, and sufficiently accurate for all purposes. Picric acid and ferrocyanide of potassium are inapplicable as precipitants, since they contain nitrogen[3]. Tannin, carbolic acid, or trichloracetic acid may be used, or Tanret's solution of potassio-mercuric iodide may be employed, bearing in mind the substances other than albumin which are liable to be thrown down. Of the available precipitants, carbolic acid and tannin appear to be the best.

The following method of determining albumin in urine is strongly recommended by M é h u. 100 c c measure of the cold urine is rendered slightly acid with acetic acid, 2 c c of concentrated nitric acid added, and the liquid thoroughly agitated. Ten c c of a mixture of 1 part by weight of crystallised carbolic acid, 1 of commercial acetic acid, and 2 of rectified spirit is next added, the liquid mixed thoroughly, and filtered after a few minutes. The filtration proceeds rapidly. The precipitate is washed with a cold 4 per

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[1] Kjeldahl's process is well adapted for this purpose. The modification of it described on page 128 is convenient when approximate results will suffice.

[2] The employment of this factor is based on the composition of albumin and its allies, which contain on the average 15 88 per cent of nitrogen, and $100 - 15\ 88 = 6\ 3$

[3] The nature of the precipitates produced by these reagents in solutions of albumin appears to be uncertain. According to some writers at least, the precipitates are actual compounds of the reagent with the proteid

cent solution of carbolic acid in water,[1] when it may
either be dried and weighed, or treated (paper and all)
by the modified Kjeldahl's method, and the nitrogen
found multiplied by 6 3 to obtain the weight of the
proteids precipitated   The presence of sugar or much
saline matter in no way affects the accuracy of this
process, but in the presence of a large proportion of
salts the addition of nitric acid becomes unnecessary

V a n N u y s and L y o n s (*Amer Chem Jour* ,
xii 336 , *Analyst*, xv 234 , xvi 7) have described a
method of determining albumin dependent on its
precipitation by a solution of tannin   This is pre-
pared, according to the method of Almén, by mixing
4 grammes of tannic acid, 8 c c of acetic acid (1 part
of glacial acid to 3 parts of water), and 190 c c of
50 per cent alcohol   Ten c c of this solution and an
equal measure of the filtered urine are mixed well,
and the liquid passed through a dry filter   In 5 c c
of the filtrate the nitrogen is then determined by
Kjeldahl's process, and by the same method the
nitrogen is determined in 5 c c of the original urine
The difference between the two results represents the
nitrogen precipitated by the tannin reagent, and this
amount multiplied by 6 3 gives the corresponding
weight of albumin and globulin in the precipitate   If
the urine contain more than 2 per cent of albuminoids
it should be diluted with an equal or double measure
of water, and the calculation modified accordingly

H O G E l l i n g e r (*Jour Prakt Chem* , [2], xlv
256) has described a method of determining albumin
in urine by means of an instrument closely allied to
Amagat and Jean's refractometer   The sample in its

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[1] According to Méhu's original directions, the precipitate is to be washed
with boiling water containing 1 per cent of carbolic acid   L R u i s a n d
(*Jour Pharm* , [5], xxix 364) finds that a very appreciable amount of albumin
is dissolved by this treatment, but that no appreciable solution occurs when
the washing is conducted as described in the text

original state is compared with the same urine from which the albumin has been removed by heat and acetic acid The results agree somewhat roughly with the gravimetric determination

Distinction and Separation of Urinary Proteids.

Until recently it was not recognised that more than one proteid body was liable to occur in urine, but it is now known that serum-globulin (paraglobulin) frequently co-exists with serum-albumin, and, according to Senator, invariably accompanies the latter substance or even exists alone Certain albumoses, proteoses or propeptones may also be present, in addition to which true peptones sometimes occur either with or without serum-albumin Traces of mucin are usually present, and fibrin and hæmoglobin may occur in certain septic and purpurous conditions

PARAGLOBULIN may be detected in urine, if present in large amount, by diluting the liquid with two measures of distilled water, rendering it faintly acid, and passing a stream of carbon dioxide On standing for twenty-four hours or more, the globulin forms a white flocculent precipitate

N o e l P a t o n (*Brit Med Jour*, 1890, page 197) has described the following method of separating and estimating paraglobulin and serum-albumin when occurring together in urine The total proteids present are first determined by Esbach's method Fifty c c measure of the urine is then rendered faintly alkaline, and powdered magnesium sulphate added until the liquid is saturated It is then allowed to stand in a warm place for twenty-four hours, when the globulin will be completely precipitated The liquid is then measured, filtered, and a portion again treated by Esbach's method (page 111) The result now obtained, after due allowance for the increased volume,

represents the albumin of the urine, and the difference between this and the figure previously obtained will be the globulin (*plus* any hemi-albumose) of the urine   The globulin and hemi-albumose ("heteroproteose") may be separated, if desired, by redissolving the precipitate produced by ammonium sulphate in a small quantity of water, and treating the solution obtained with ten times its measure of absolute alcohol   The resultant precipitate is collected and digested with cold absolute alcohol for a week or ten days   The liquid is then filtered, when a residue will consist of globulin, and the hemi-albumose will be found in the filtrate

In the urine of a person recovering from a prolonged attack of diarrhœa, Paton found 2 per cent of total proteids, of which 1 92 was globulin , and in another case 3 82 of total proteids, of which 3 73 was globulin, which was obtained in elongated rhombic crystals

Instead of employing Esbach's method, A O s t (*Chem Centralb* , 1884, page 500) observes the optical activity of the urine before and after saturation with magnesium sulphate

An alternative plan is to precipitate the globulin and albumin together by boiling the faintly acidulated urine, and estimate the nitrogen in the washed precipitate by the a u t h o r ' s modification of Kjeldahl's method (page 128)   In another portion of the urine the globulin is precipitated by saturating the liquid with magnesium sulphate, the precipitate collected and washed with magnesium sulphate solution, and the contained nitrogen estimated by the modified Kjeldahl's process   The difference between the two results is the nitrogen corresponding to the albumin of the urine

According to S e n a t o r , paraglobulin occurs in urine in cases of lardaceous disease of the kidneys, and has also been found in excess in the intense hyperæmia

resulting from poisoning by cantharides, and in functional albuminuria associated with marked disturbance of the digestive organs   The greater the proportion of paraglobulin present, the more unfavourable appears the diagnosis in Bright's disease   When blood is present in urine, as in nephritis after scarlet fever, there is a large increase in the proportion of paraglobulin   N o e l   P a t o n (*Brit Med Jour* , ii , 1890, page 196) finds the ratio of globulin to albumin to vary enormously (from 1 0 6 to 1 39).   It varies much during the day, and in such an erratic manner that no conclusions can be drawn

ALBUMOSES or PROTEOSES

Of late years a series of bodies, intermediate in characters and composition between the albumins and peptones, have been isolated, and some of these have been proved to exist in certain forms of pathological urine   Thus G e r r a r d has recently shown (*Pharm Jour* , [3], xxiii 261) that in the milk-treatment of albuminuria no albumin coagulable by heat exists in the urine, but that nitric acid gives a precipitate soluble in excess, or on warming, reappearing on cooling , and saturated brine a flocculent precipitate increased by the addition of acetic acid   These reactions are characteristic of the form of proteose called hetero-albumose or hetero-proteose, the presence of which in urine is said to be an indication of approaching nephritis   The same substance has been found in cases of osteo-malacia and atrophy of the kidneys, and in the urine of persons who have been rubbed with petroleum

For the detection of hetero-albumose, T y s o n acidifies the urine with a few drops of acetic acid, and adds one-sixth of its volume of saturated brine, boils, and filters   Albumin and globulin are precipitated If the filtrate after cooling gives a precipitate on

further addition of brine, which dissolves on heating and reappears on cooling, the presence of albumose is indicated

For the distinction of the proteoses liable to occur in urine, and their separation from co-occurring proteids, the following plan may be used. It is taken, with slight modifications, from W D Halliburton's *Text-book of Chemical Physiology and Pathology*

Bring the urine to a faintly acid condition by cautious addition of acetic acid or dilute caustic alkali (as the original reaction to litmus may indicate), boil for a minute or so, and filter			
PRECIPITATE consists of *albumin* and *globulin* These may be separated by saturating another portion of the faintly acidulated urine with powdered magnesium sulphate, which precipitates *globulin*, *proto albumose*, and *hetero-albumose* From the filtrate, albumin will be precipitated on boiling, while deutero albumose and peptones remain in solution	FILTRATE, allow to cool, saturate thoroughly with powdered ammonium sulphate, and filter		
	PRECIPITATE consists of albumoses Wash with a saturated aqueous solution of ammonium sulphate Redissolve the washed precipitate in a minimum quantity of water Faintly acidulate the solution with acetic acid, saturate it with common salt, and filter		FILTRATE contains *peptones* only, recognisable by xanthoproteic and biuret reactions, as on page 123
	PRECIPITATE consists of *primary albumoses* Wash with saturated brine, redissolve by adding water, and dialyse the solution	FILTRATE may contain *deutero-albumose*, precipitated by saturating the solution with ammonium sulphate, and recognisable by other reactions on page 123	
	PRECIPITATE consists of *hetero albumose*, which may be further identified by reactions on page 120	FILTRATE may contain *proto albumose*, precipitable by excess of alcohol (10 1)	

Traces of albumoses are liable to be formed by the action of the boiling acidulated liquid on the albumin and globulin A sharper separation can be effected by treating the urine with ten times its volume of

strong alcohol, which precipitates all the proteids
The precipitate is rinsed off the filter with absolute
alcohol, and left in contact with the alcohol for five to
ten weeks   This treatment coagulates the albumin
and globulin without affecting the albumoses or
peptones   The supernatant alcohol is poured off, the
remainder evaporated at a temperature not exceeding
40° C (= 104° F), and the residue treated with water,
which dissolves the albumoses and peptones, leaving
the albumin and globulin insoluble

PEPTONES are now known to occur in the urine
under a great variety of pathological conditions,
especially in acute febrile diseases and nervous com-
plaints, and they probably exist in traces in normal
urine   The only reliable method of distinguishing
and separating peptones from co-occurring proteids
appears to be that of S H C M a r t i n (*Brit Med
Jour* , 1 , 1888, page 842)   This consists in saturat-
ing the urine, faintly acidulated with acetic acid, with
ammonium sulphate   The powdered salt is added
gradually to the urine till no more is taken up   The
precipitate of proteids rises to the surface of the
liquid and can readily be separated by filtration [1]   By
this treatment any albumin, globulin, or proteose is
completely precipitated, whatever the reaction of the
liquid, while any peptone remains in solution

Peptones present the closest similarity in their pro-

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[1] If the precipitate be washed twice with a cold saturated solution of
ammonium sulphate, and then redissolved on the filter in distilled water, a
solution is obtained in which the proteids are easily differentiated   Thus, on
saturating the liquid with magnesium sulphate, the globulin is precipitated,
while the albumin can be detected in the filtered liquid by its property of
coagulating when heated to 73° C , after slightly acidulating the solution with
acetic acid   Hemi albumose is precipitated at 43° to 50° C , the precipitate
being soluble in a few drops of a weak acid, and it is precipitated in the
absence of acids, which albumin and globulin are not   It also gives a pink
reaction with the biuret test, and with nitric acid a precipitate which dis
solves on heating and reappears on cooling   It is likewise precipitated from a
solution faintly acidulated with acetic acid by potassium ferrocyanide, and
by saturating its solution with magnesium sulphate

perties and reactions to the proteoses, and especially to the body called deutero-proteose or deutero-albumose Peptones differ from deutero-proteose in not being precipitated on saturating the solution with ammonium sulphate, and in giving no precipitate with nitric acid under any conditions Deutero-proteose, on the other hand, is precipitated by nitric acid after a considerable quantity of common salt has been added to the liquid This precipitate dissolves on heating the liquid containing it, but reappears on cooling

Both peptone and deutero-albumose are precipitated, but not coagulated, by alcohol They are not precipitated by boiling, nor by cupric sulphate, but are precipitated by phospho-molybdic, phospho-tungstic, picric acid, tannin, or potassio-mercuric iodide They resemble other proteids in yielding a yellow coloration on boiling with nitric acid, becoming brownish on adding excess of ammonia (the " xantho-proteic reaction "), and by the pink or rose-red coloration obtained on adding excess of caustic alkali, followed by a few drops of a very dilute solution of cupric sulphate (" biuret reaction")

These two reactions can be employed for the detection of peptones in the filtrate from the precipitate produced by saturating the urine with ammonium sulphate, as above described

Where mucin and albumin are already absent, it is stated that peptones may be detected by treating 50 c c of the original urine with 5 c c of hydrochloric acid and precipitating the warm liquid with sodium phospho-tungstate The supernatant liquid is decanted, and the resinous precipitate washed twice with water containing 0 5 c c of caustic soda solution of 1 16 specific gravity, which dissolves it The resultant solution is warmed till a greenish turbidity

is produced, allowed to cool, and a 1 per cent solution of cupric sulphate added drop by drop   In presence of a peptone, a red coloration is produced, which is rendered more evident by filtering the liquid

Roux (*Jour Pharm*, [5], xxv 544) proposes to determine the peptones in urine volumetrically   The sample is freed from albumin and reducing compounds, and a decinormal Fehling's solution added until the colour changes through light blue, blue-violet, lilac, and rose-purple, to a greyish tint   One c c is said to represent 0 004 gramme of peptone

MUCIN is the chief constituent of the mucus derived from the renal and urinary passages, and is probably the source of the so-called " animal gum " found in the urine by Landwehr   It occurs very commonly (according to Sir G Johnson, invariably) in traces even in normal urine, and in larger amount in urinary catarrh and other affections of the urinary organs Mucin is slightly soluble in neutral or alkaline urine, but is precipitated on adding acetic acid, and is insoluble in excess of the precipitant   In many of its reactions it resembles albumin, for which it is apt to be mistaken, but is not coagulated by heat   It is precipitated by alcohol, alum, dilute mineral acids, and by certain organic acids, including acetic and citric   If urine containing mucin be poured on to the surface of acetic acid *saturated* with salt, or on to a strong solution of citric acid, a cloud appears at the junction of the two layers   On the other hand, mucin is soluble in saline solutions of moderate strength, and P u r d y actually adds brine to urine in quantity sufficient to raise its density to 1035, in order to prevent the precipitation of mucin when acetic acid is subsequently added   In the nitric acid test for albumin the haze due to mucin appears above and distinct from that produced by albumin

Salkowski and Leube test for mucin by treating the urine with two measures of nearly absolute alcohol, separating the precipitate by filtration, and redissolving it in water The resultant solution gives with acetic acid a cloud which is insoluble in excess, but soluble in hydrochloric or nitric acid Mucin gives the violet biuret reaction with caustic alkali and cupric sulphate, and is completely precipitated by lead acetate

Mucin may be separated from pus by precipitating the latter by mercuric chloride On adding acetic acid to the filtrate the mucin is precipitated Pus is characterised by forming a gelatinous mass with alkalies, which reagents give no reaction with mucin

There are several varieties of mucin They all appear to have the constitution of glucosides, being compounds of a proteid (probably variable, but generally a globulin) with animal gum, which, by boiling with dilute sulphuric acid, yields a reducing unfermentable sugar

In certain forms of liver-disease it has been found that the urine gives precipitates which may be mistaken for those of albumin, but which are in reality *bile-pigments* It has been found, however, that these can be got rid of by previous treatment with acetic or a dilute mineral acid Giocco, therefore, recommends that all samples of urine likely to contain such matters should be first treated with 2 or 3 per cent of concentrated acetic acid, set aside in a cool place for two or three hours, and then filtered, before applying the ordinary tests The precipitate thus formed by the addition of acetic or dilute mineral acids is soluble in alcohol, and does not give the biuret reaction

THE NITROGENISED CONSTITUENTS OF URINE

THE determination of the nitrogen contained in urine is often of great physiological and pathological interest, since the whole of the nitrogen contained in the effete nitrogenised tissues and in the food digested is ultimately eliminated by the kidneys

About 90 per cent of the total nitrogen contained in normal human urine exists in the form of u r e a, the remainder being divided between u r i c a c i d, h i p p u r i c a c i d, x a n t h i n e, c r e a t i n i n e, &c [1] In the urine of herbivorous mammals the uric acid is replaced by hippuric acid, while the nitrogen of birds and reptiles is eliminated chiefly in the form of uric acid instead of as urea

As urea is the predominant nitrogenous constituent of normal human urine, it is evident that for many purposes its determination will afford sufficient infor-

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[1] The result of a large number of observations by R u s s e l l and W e s t (*Proc Royal Soc*, xxx 439) on various cases of disease was to prove that the relation of the ureal to the total nitrogen of urine is approximately constant, except in rare cases of acute yellow atrophy of the liver, and even in these it is doubtful whether the observed replacement of the urea by leucine and tyrosine is a constant phenomenon   In a case of acute fatty atrophy of the liver the urea was still normally formed, while leucine and tyrosine were absent   The following table shows the percentage of the total nitrogen existent as urea, according to the observations of Russell and West —

Pneumonia (6 cases), 90 per cent, jaundice (Case 1), 85 7, jaundice (Case 2), 90 2, albuminuria (2 cases), 86 0, collected cases, 93 8, dieted cases, 90 1, and mean of all, 89 3 per cent   The mean, excluding the jaundice and albuminuria cases, was 91 3 per cent

mation as to the amount of nitrogen passing away in the urine

Albumin and its allies are not present in normal urine, at least not in estimable amount, and their detection and determination in the pathological excretion have already been fully considered (page 105 *et seq*)

Determination of the Total Nitrogen in Urine

In certain cases it is desirable to ascertain the total amount of nitrogen existing in urine, without differentiating between the different modes of combination The total amount of nitrogen is best ascertained by the well-known K j e l d a h l method, which is based on the fact that nearly all forms of nitrogenised organic matter yield their nitrogen in the form of ammonia when heated strongly with concentrated sulphuric acid Urea readily undergoes this conversion, and the decomposition of uric acid presents no difficulty Albumin is split up primarily into glycocine, leucine, tyrosine, and probably other amido-compounds, but these, on further heating with strong sulphuric acid, yield all their nitrogen in the form of ammonia G u n n i n g finds the destruction of the organic matter to be much facilitated by adding some solid potassium sulphate, which raises the boiling point of the sulphuric acid [1]

The conversion of the nitrogen into ammonia having been effected by the foregoing treatment, excess of caustic soda is added and the ammonia distilled off, an addition of sulphide of alkali-metal

[1] Potassium permanganate has been employed, but it is now recognised that its use is unnecessary and liable to lead to loss from oxidation of the ammonia formed W F K S t o c k effects rapid and complete oxidation of the organic matter by adding manganese dioxide to the hot acid This plan is not available when the ammonia formed is to be decomposed with hypobromite, as described in the text The use of mercury or mercuric oxide, which is a convenient means of effecting oxidation, results in the formation of a mercur ammonium compound not decomposed by the reagent

being made if mercury has been employed, in order to ensure the decomposition of mercur-ammonium bases

The ammonia in the distillate is finally determined by titration with standard acid, and the amount found calculated into its equivalent of nitrogen

The estimation of nitrogen by this process requires the observance of numerous precautions to ensure accuracy

In a modified process, which presents fewer difficulties than the foregoing, and gives results sufficiently accurate for the ordinary purposes of urinary analysis, the nitrogen is first converted into ammonia by strongly heating with sulphuric acid, and the ammonia then decomposed by sodium hypobromite, the nitrogen evolved being measured in the form of gas   When treated with an alkaline hypobromite, ammonia compounds are decomposed in accordance with the following equation —

$$3NaBrO + 2NH_3 = 3NaBr + 3H_2O + N_2$$
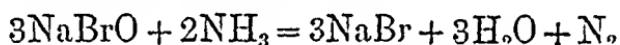
This reaction, which was first applied by W Knop, under favourable conditions occurs very promptly and completely, but in order to adapt the method to the requirements of urinary analysis, the manipulative details require careful arrangement   They have been thoroughly worked out in the author's laboratory, and by operating in the following manner fairly good determinations of the total nitrogen of urine can be obtained in about one hour [1]

Twenty-five c c of the urine to be examined should be treated in a porcelain basin with 10 c c of strong sulphuric acid, and the liquid kept gently boiling until the volume is reduced to about 10 c c and

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[1] A process on the same lines has been described by Petit and Monfet (*Jour Pharm und Chem* , 1893, page 297), but their method of manipulating is different in many respects from that employed by the author   Both modifications are liable to give results below the truth

white fumes of sulphuric acid are evolved[1] The liquid is then allowed to cool, and carefully trans- ferred to a pear-shaped flask, the basin being rinsed with a few drops of water The flask is placed in an inclined position, to prevent loss by spurting, and the contents kept in gentle ebullition   If excessive frothing occur, it may be moderated by adding a small fragment of paraffin-wax (candle)   When the frothing has ceased, about 5 grammes of potassium sulphate should be added and the flask heated strongly until the liquid is colourless or only a very pale yellow   The contents of the flask are then allowed to become cold, when about 20 c c of water is added very cautiously and a few drops at a time, agitating the liquid by a rotatory movement between each fresh addition   A highly concentrated solution of caustic soda, made by dissolving the alkali in about an equal weight of water, is now added gradually with constant agitation, until the sulphuric acid is nearly neutralised   This point may be ascertained by means of litmus-paper, or a few drops of litmus or phenol-phthalein solution may be added to the contents of the flask

The neutralised liquid, which will measure about 80 c c , is now diluted to exactly 100 c c with water, and thoroughly mixed by agitation   Ten c c of the solu-

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[1] As a rule, the quantity of sulphuric acid prescribed is amply sufficient for the decomposition of the solids of 25 c c of urine   In the case of highly saccharine urine, however, the sugar chars and forms a black pasty mass, which cannot be readily transferred to the flask   In such a case, a further addition of sulphuric acid (5 to 10 c c) should be made, and the heating continued till the greater part of the carbonaceous matter is oxidised   It is important in all cases to avoid the use of an excessive amount of sulphuric acid, or so large an amount of soda must be employed to neutralise it, and so large a volume of water added to retain the salts in solution, that the measure of the neutralised liquid cannot be kept within 100 c c , or indeed within any reasonable limits   On the other hand, less than 10 c c of acid is an inconveniently small volume to heat and manipulate   Hence it is desirable to adhere to the quantities of urine and acid prescribed in the text, and take an aliquot part of the neutralised liquid for treatment with hypobromite.

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tion, representing 2 5 c c of the original urine, is now treated with the alkaline hypobromite reagent used for the determination of urea (page 141)   The manipulation is the same as is described in the section on " Urea," and one of the forms of apparatus therein referred to may be employed   By far the most convenient apparatus for the purpose is, however, that shown in fig 7   Ten c c of the neutralised solu-
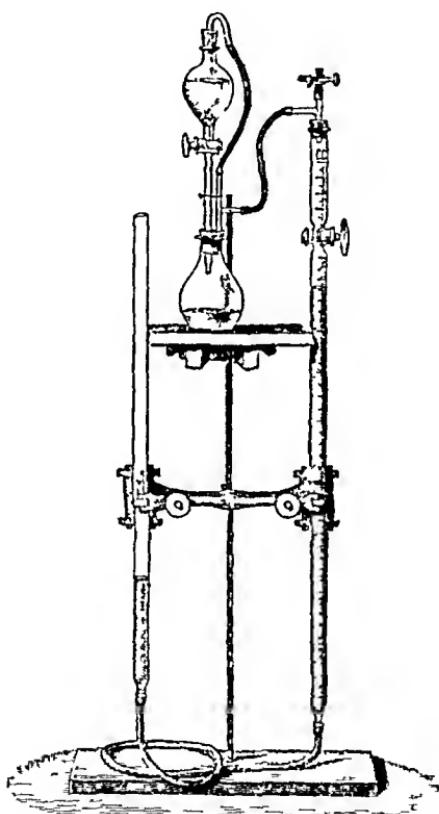
Fig 7 —Nitrogen Evolution Apparatus

tion from the sulphuric acid treatment being placed in the flask, 25 c c of the hypobromite reagent should be poured into the separator, and the connections made as shown in the figure   The nitrometer should be filled to the tap with water or (preferably) brine, or, for very accurate experiments, mercury may be advantageously used   The apparatus being

adjusted, and the clip at the top of the nitrometer-cup having been momentarily opened to equalise the pressure in the cup with that in the flask, the tap of the nitrometer is opened, and the hypobromite solution then allowed to flow gradually into the flask After adding about 10 c c , the separator-tap should be closed and the flask agitated A further addition of hypobromite is then made, the flask again agitated, and this treatment repeated until no further evolution of nitrogen takes place As a rule, 10 c c of the reagent is sufficient to complete the reaction, which occurs very promptly and completely [1] The flask is now allowed to acquire the temperature of the room, when the liquid in the nitrometer-tube is brought to the same level with that in the reservoir-tube, and the volume of nitrogen read off If less than 20 c c of gas has been evolved, the process may be advantageously repeated on 20 c c or more of the neutralised liquid from the sulphuric acid treatment

From the number of cubic centimetres of nitrogen evolved, v, the corresponding weight in milligrammes, W, may be calculated by the following formula, in which p represents the barometric pressure in millimetres, w the tension of aqueous vapour at

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[1] The following figures, obtained in the author's laboratory by G B Brook, show that the process gives tolerably good, but somewhat low, results with solutions of pure ammonium sulphate, and that the reaction is fairly complete unless the dilution is excessive

	Volumes of Solutions employed					Percentage of Total Nitrogen evolved
	Soda	Bromine	Water	Am Sulphate	Total	
A	25 c c	2 5 c c	none c c	5 c c	32 5 c c	100 0
B	25 ,,	2 5 ,,	20 ,,	5 ,,	47 5 ,,	98 1
C	25 ,	2 5 ,,	30 ,,	5 ,,	62 5 ,,	97 1
D	25 ,,	2 5 ,,	50 ,,	5 ,,	82 5 ,,	92 7

If preferred, the hypobromite reagent may be placed in the flask and the neutralised solution added to it In this case, a layer of water should be floated on the liquid in the separator, so as to rinse it completely into the flask

the temperature at which the gas was measured, and t the temperature in centigrade degrees —

$$W = \frac{v \times (p - w)}{273 + t} \times 1\,251$$

When strictly accurate results are not required, the corrections for temperature, pressure, and tension of aqueous vapour may be omitted and the calculation much simplified   Thus, the volume occupied by 28 milligrammes ($= 0\,028$ gramme) of moist nitrogen at $16°$ C ($= 61°$ F) and 762 millimetres ($= 30$ inches) pressure is 24 c c   From this it follows that the grammes of nitrogen contained in 100 c c of urine can be calculated by the following equation, in which G represents the number of c c of gas evolved, and U the volume (in c c) of the original urine represented by the neutralised liquid used —

$$N = \frac{G \times 28 \times 100}{U \times 24 \times 1000} = \frac{G \times 7}{U \times 60}$$

Thus if the gas evolved from a measure of the neutralised liquid corresponding to 5 c c of the original urine measured 38 2 c c , the sample contained 0 891 gramme of nitrogen per 100 c c

$$N = \frac{38\,2 \times 7}{5 \times 60} = \frac{267\,4}{300} = 0\,891$$

This figure, multiplied by 4 375, will give the grains of nitrogen per fluid ounce of the urine , or, if divided by the specific gravity of the sample (water $= 1\,000$), the actual percentage by weight of nitrogen contained in the urine will be obtained

It is evident that as Kjeldahl's process is applicable to the determination of the nitrogen of urea, uric acid, creatinine, albumin, &c , as they exist in urine, it is equally applicable to its determination in these substances when in an isolated state   Thus the nitrogen

contained in a precipitate of *uric acid* may be ascertained, and the weight of uric acid itself calculated therefrom , compounds of *creatinine* may be subjected to the treatment and the base thus determined[1] , or the *albumin* may be precipitated from 100 c c of urine, washed, and treated (together with the filter containing it) with strong sulphuric acid [1]

A volume of 24 c c of moist nitrogen, measured at the ordinary pressure and temperature, corresponds to —

0 028 gramme of		Nitrogen ,
0 034	,,	Ammonia ,
0 060	,,	Urea ,
0 084	,,	Uric acid ,
0 358	,,	Hippuric acid , or
0 164	,,	Albumin

Urea. Carbamide. CH_4N_2O , or, $CO(NH_2)_2$

Urea exists ready-formed in the urine of mammals, and in blood, milk, and other animal fluids It was first prepared synthetically by L i e b i g and W o h l e r in 1828, being the first of the natural organic bodies obtained by a synthetic process [2]

Urea forms transparent, colourless, four - sided, anhydrous prisms (fig 8) It is somewhat hygroscopic Urea is odourless, and possesses a cooling saline taste, resembling that of nitre When heated

[1] The evolution-method cannot be recommended in the case of creatinine Albumin requires prolonged treatment with sulphuric acid to effect complete conversion of the proximate products of decomposition into ammonia

[2] This classical discovery affords an interesting example of re arrangement of the atoms in the molecule Both ammonium cyanate and urea have an elementary composition corresponding to the empirical formula — CH_4N_2O On evaporating an aqueous solution of a m m o n i u m c y a n a t e at the temperature of boiling water, the salt suffers molecular change into urea, according to the equation — $CN\ O(NH_4) = CO(NH_2)_2$ The converse reaction occurs when an aqueous solution of urea is heated with silver nitrate A white precipitate of silver cyanate is formed, soluble in boiling water, while the solution is found to contain ammonium nitrate — $CO(NH_2)_2 + AgNO_3 = CN\ OAg + (NH_4)NO_3$

to 132° C it melts, and at a higher temperature decomposes with evolution of a m m o n i a and a m m o- n i u m c y a n a t e, leaving a residue of c y a n u r i c a c i d, $C_3H_3N_3O_3$, which bears a much stronger heat without change

Urea is soluble in an equal weight of cold water, and in a much less quantity of hot   It is also readily soluble in alcohol, and dissolves in amylic alcohol, but it is nearly insoluble in ether, and quite so in chloro- form and volatile oils

At the ordinary temperature, an aqueous solution of pure urea shows no tendency to change, and is not decomposed by boiling, but when heated with water

Fig 8 —CRYSTALS OF UREA—a, Quadrangular prisms, b, Indefinite crystals, as deposited from alcoholic solutions

under pressure urea undergoes hydrolysis, with for- mation of ammonium carbonate, $CH_4N_2O + 2H_2O = (NH_4)_2CO_3$ In the urine, where the urea is associated with putrescible organic matter, it readily undergoes a similar change, which is the cause of the alkaline reaction of putrid urine   The ammoniacal fermenta- tion of urine has been found to be due to the action of an organised ferment (*Torula ureæ*) in the urine This change is set up by contact with the stomachs of men, dogs, or rabbits, and has often been occa-

sioned in the bladder by the introduction of a septic catheter

Urea also yields ammonia when fused with caustic alkali or ignited with soda-lime, a carbonate being formed at the same time   When heated with a strong mineral acid, urea similarly forms an ammoniacal salt, carbon dioxide being evolved

Pure concentrated nitric acid combines with urea without decomposing it, but if the acid contain nitrous acid the urea is resolved into water, nitrogen, and carbon dioxide, according to the following equation, the reaction, however, being far from complete —

$$CH_4N_2O + N_2O_3 = 2H_2O + 2N_2 + CO_2$$
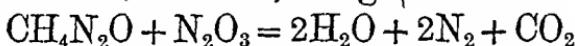
Chlorine, bromine, hypochlorites, and hypobromites decompose solutions of urea with evolution of nitrogen   The best practical method of determining urea in urine is based on this reaction (page 138)

The basic character of urea is well-marked, although its solutions exhibit no alkaline reaction to litmus   It forms a series of well-defined salts, some of which crystallise readily   Many of them are decomposed by water

Urea Nitrate, $CH_4N_2O \, HNO_3$, separates in crystals when moderately strong nitric acid is added to a concentrated aqueous solution of urea, and the liquid cooled   The compound forms brilliant white scales or plates, or, if the deposition is slow, prismatic crystals When nitric acid and urea are brought together on a microscope-slide, and the reaction observed under a low power, the formation of obtuse rhombic octahedra is first noticed, the angles being *constantly* 82° These octahedra change to rhombic and hexagonal tables, either separate or superposed (see fig 9, *a*), but also having angles of 82°   For the formation of nitrate of urea from normal urine, it is sufficient to concentrate the liquid to about one-fourth of its

volume, filter after cooling from the precipitated urates, &c, and add nitric acid to the cold filtrate Nitrate of urea is unalterable in the air   It is readily soluble in water, forming a solution of acid reaction and taste   It is also soluble in alcohol, but only very slightly in presence of nitric acid   Oxalic acid precipitates urea oxalate from concentrated solutions of the nitrate
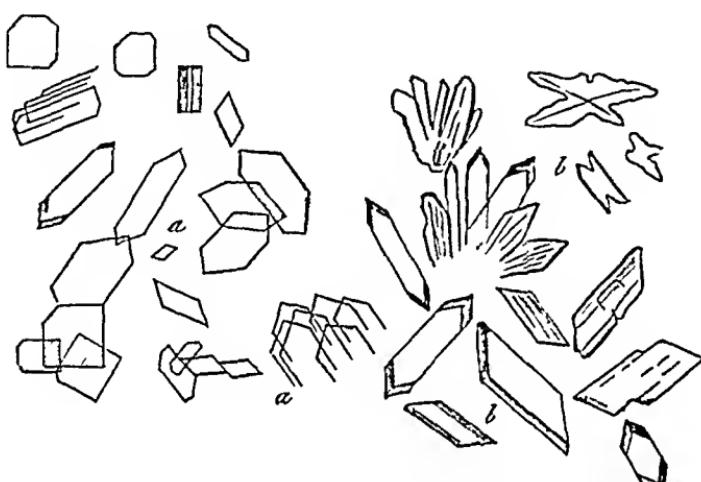
Fig 9 —*a*, UREA NITRATE, *b*, UREA OXALATE

Urea Oxalate, $(CH_4N_2O)_2 \, C_2H_2O_4$, is readily formed on mixing concentrated solutions of urea and oxalic acid   From urine it may be prepared by adding oxalic acid to the concentrated and filtered liquid Urea oxalate forms thin crystalline plates (see fig 9, *b*), usually grouped together, but sometimes in well-formed separate crystals   Its microscopic appearance is not unlike that of the nitrate of urea, but the forms are less characteristic, and the angles are different Oxalate of urea is soluble with difficulty in cold water, but dissolves readily at a boiling heat   It is less soluble in a solution of oxalic acid than in pure water   The salt dissolves in 62 parts of alcohol, but is quite insoluble in amylic alcohol   Hence, if a solution of urea in amylic alcohol (such as will result

from evaporating urine to dryness, heating the residue with amylic alcohol, and filtering) be treated with a cold saturated solution of oxalic acid in amylic alcohol, urea oxalate is precipitated in small crystals By warming the liquid until the crystals are redissolved and allowing it to cool, the salt is obtained in a state fit for microscopic examination The process may be modified by treating the solution of urea in amylic alcohol with one of oxalic acid in anhydrous ether Precipitation takes place abundantly and quickly, but the crystals are usually small and imperfect The oxalic acid may be added in powder, the liquid heated and thoroughly cooled, and the excess of oxalic acid removed from the precipitate by treatment with anhydrous ether The method is capable of being employed quantitatively The amylic alcohol used in the process must not develop a red or brown colour with oxalic acid, and should be free from water and ethylic alcohol

Urea Phosphate, CH_4N_2O,H_3PO_4, forms large, very soluble, rhombic crystals on evaporating pig's urine, or mixed solutions of urea and phosphoric acid

A compound of urea with s o d i u m c h l o r i d e, of the formula $CH_4N_2O,NaCl,H_2O$, separates in brilliant rhombic crystals when mixed solutions of urea and common salt are evaporated It sometimes crystallises from concentrated human urine

On mixing a solution of urea with one of neutral mercuric nitrate, a white flocculent precipitate is obtained This has a composition dependent on the concentration of the liquid, containing, according to the conditions of its formation, 1, $1\frac{1}{2}$, or 2 molecules of mercuric oxide to 1 of urea If, however, the addition of the mercuric nitrate be continued as long as precipitation occurs, and sodium carbonate be added from time to time to neutralise the nitric

acid set free, the precipitate has the composition $CH_4N_2O,2HgO$ The end of the reaction is indicated by the yellow colour developed from the formation of basic nitrate of mercury   L i e b i g's method of determining urea, now chiefly of historical interest, was based on this reaction.

Urea is not precipitated by a solution of mercuric chloride   The addition of mercuric nitrate to a soluble chloride results potentially in the formation of mercuric chloride   As sodium chloride 'is present in urine, mercuric nitrate produces no precipitate of Liebig's compound in that liquid until sufficient has been added to react fully with the chloride present   On this fact Liebig based a method for determining chlorides in urine

Mercuric acetate gives no precipitate with urea in the cold, and the separation is very incomplete on boiling

For the recognition of urea in a weak aqueous solution B l o x a m has suggested the following method — If a nitrate be present, add a few drops of ammonium chloride solution, but if absent, acidulate the liquid with hydrochloric acid   Evaporate the solution to dryness in a watch-glass, and heat the residue cautiously as long as thick white fumes are evolved   Dissolve the cooled residue in a drop or two of ammonia, add a drop of barium chloride, and stir   If urea was present, crystalline streaks of b a r i u m c y a n u r a t e will be formed in the track of the glass rod

DETERMINATION OF UREA

Various methods have been devised for the determination of urea in urine, but those dependent on the measurement of the nitrogen gas evolved by its decomposition are by far the most convenient, and sufficiently accurate for ordinary purposes   They are

based on the reaction between urea and a strongly alkaline solution of hypobromite of sodium, whereby sodium bromide, water, carbon dioxide, and free nitrogen are produced, according to the following equation —

$$3NaBrO + CH_4N_2O = 3NaBr + 2H_2O + CO_2 + N_2$$
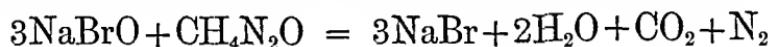
The carbon dioxide (carbonic acid) gas is absorbed by the excess of caustic alkali employed, so that, under the conditions of the experiment, pure nitrogen gas is evolved

According to the great majority of observers, the reaction of cold hypobromite solution of the strength commonly employed results in the evolution of from 92 to 93 per cent of the nitrogen existing in the urea present [1]   The suppressed nitrogen has been found to suffer conversion into cyanate   On heating the liquid, some further evolution of gas occurs, but the theoretical production is never realised, and under some circumstances there is a tendency to error from evolution of oxygen   In presence of cane-sugar, a more complete evolution of the nitrogen occurs, and hence C Méhu (*Bull Soc Chim*, [2], xxxiii 410, and *Jour Chem Soc*, xxxviii 681) has proposed always to add 10 parts of cane-sugar for each 1 of urea supposed to be present   Glucose induces a still more perfect evolution of the nitrogen, but is said to be apt to occasion the liberation of traces of gas even in the absence of urea   In consequence of the peculiar action of glucose, the evolution of nitrogen on treating diabetic urine with hypobromite reaches 99 per cent of the total nitrogen present in the urea   Even in normal urine, the evolved nitrogen bears a greater

[1] According to T G Wormley (*Chem News*, xlv 27), under favourable conditions the whole of the nitrogen of urea is evolved as gas   J R Duggan (*Amer Chem Jour*, iv 47, and *Jour Chem Soc*, xlii 778) states that fully 99 per cent of the nitrogen is evolved as gas if 5 c c of the urine be first mixed with 20 c c of a solution of 20 grammes of caustic soda in 100 c c of water, and 1 c c of bromine is subsequently added

proportion to the total amount present in the urea than is the case when pure solutions of urea are operated on This fact is commonly attributed to the liberation of nitrogen from the uric acid, creatinine, and other urinary constituents, which, though present in but small amount relatively to the urea, are able to exert a sensible influence on the proportion of gas evolved For ordinary purposes, the error due to this cause is wholly unimportant, and it has even been contended that, as the usual object of determining urea is to obtain a measure of the nitrogenous waste, all nitrogenised constituents of the urine should, as far as possible, be determined Of course this is directly effected by determining the total nitrogen in the manner described on page 128

M é h u points out that the uric acid present in urine is rarely more than 2 per cent of the urea, and the creatinine still less, and as these bodies only evolve a portion of their nitrogen when treated with hypobromite they are incompetent to produce the whole of the effect ascribed to them He attributes the better yield of nitrogen obtained from normal urine to the extractive matter present, which acts more or less like sugar Hence Méhu recommends the addition of sugar in every case, so as to ensure the evolution of practically the whole of the nitrogen in the gaseous state.

G Esbach (*Bull Soc Chim*, [2], xxiv 632) states that pure glucose solution, whether boiling or not, evolves a small quantity of gas when treated with the hypobromite reagent, but that cane-sugar yields no gas He finds that in aqueous solutions of urea, the excess over the 92 per cent normally evolved varies with the quantity and nature of the sugar added, the strength of the urea solution, and composition, especially the free alkali of the hypobromite

reagent, the volume of gas being greater the more alkali there is present   For the proportions of glucose present in diabetic urine, the excess of nitrogen over 92 per cent is sensibly proportional to the mass of the sugar, but this does not hold good for large quantities Urea *added* to a true diabetic urine is stated to evolve only 92 per cent of its nitrogen   Esbach concludes that sugar should not be added to urine before applying the hypobromite process

A very large number of determinations of urea by the hypobromite method have been made in the author's laboratory, the process being varied in many cases with the view of ascertaining the best conditions under which to work   The results have shown that the method is by no means so constant as generally supposed, and that it is liable to give low results from undiscovered causes   In presence of glucose or cane-sugar great evolution of heat occurs, and it is probably this phenomenon which is the cause of the higher results obtained in presence of sugar   Dilution of the hypobromite reagent with water, or the use of less bromine, did not greatly affect the results, but a large excess of bromine was prejudicial

The hypobromite solution employed for the decomposition of urea is prepared by dissolving 100 grammes of good caustic soda in 250 c c of water, and thoroughly cooling the liquid , 25 c c measure of bromine is then added, and the resultant solution preserved in a cool place [1]   The reagent does not keep very well, in consequence of the gradual occurrence of the reaction — $3NaBrO = 2NaBr + NaBrO_3$ Hence it is preferable to prepare the solution, when required, by mixing 25 c c of the caustic soda solution with 2 5 c c of bromine   Considerable variations in the strength of the reagent do not materially affect the results

[1] Or $3\frac{1}{2}$ oz of caustic soda, 9 oz of water, and $1\frac{3}{4}$ fluid ounce of bromine

Various forms of apparatus have been devised for effecting the reaction and collecting the evolved nitrogen, and a suitable arrangement is easily extemporised   Of the special forms, that devised by A W G e r r a r d (*Pharm Jour* , [3], xv 464) is among the best, when no extreme degree of accuracy is aimed at   Gerrard's apparatus (fig 10) consists of a graduated tube, which is connected with a second tube, serving   as a reservoir, by means of india-rubber tubing   The top of the graduated tube is closed by a
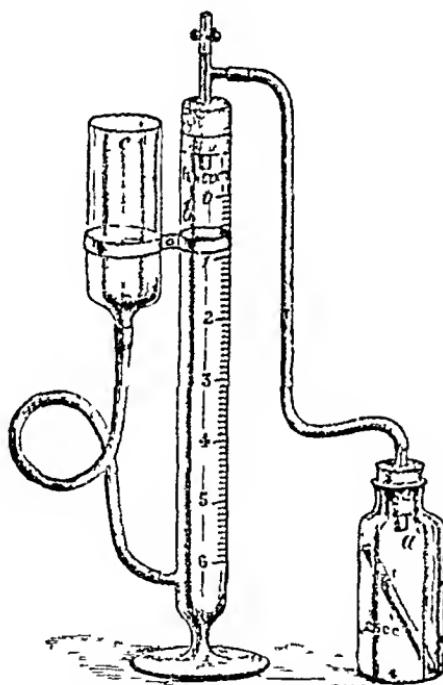
Fig 10 —GERRARD'S UREOMETER

caoutchouc stopper, through which passes a T-tube, one orifice of which is fitted with a short piece of india-rubber tubing closed by a clip, while the other communicates by a second piece of tubing with a bottle fitted with a perforated cork   In making the test, 25 c c of the hypobromite reagent should be poured into this bottle, and then a small test-tube containing 5 c c of the sample of urine cautiously placed in

it in such a manner as to avoid any contact between the urine and the reagent The bottle is now connected with the graduated tube in the manner shown in the figure, the clip opened, and water poured into the reservoir-tube until, on suitably adjusting its height, the water stands at the zero-point in the measuring tube and at the same level in the reservoir, taking care that when this is effected but little water remains in the latter tube [1]

The clip is then closed, and the bottle is tilted in such a manner as to allow the urine to mix gradually with the hypobromite solution, the bottle being gently agitated to promote the evolution of gas, which commences immediately and is complete in a few minutes After five minutes, or preferably ten, the water in the measuring and reservoir tubes are brought to the same level by lowering the latter, when the volume of gas is read off [2] In Gerrard's apparatus the tube is so graduated as at once to show the percentage of urea contained in the urine If the urine contain more than 3 per cent of urea, it is necessary to take 2 5 instead of 5 c c, dilute it with an equal measure of water, and double the result obtained With normal urine, the volume of nitrogen evolved under the foregoing mode of treatment is only 92 per cent of that which would be yielded if the reaction formulated on page 139 were the only one which took place , but as a fact, some $7\frac{1}{2}$ to 8 per cent is suppressed Gerrard's apparatus is so graduated as to allow for this loss, and hence gives correct results when non-saccharine urine is under examination But, in the case of urine containing much sugar, fully 99 per cent of the ureal nitrogen

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[1] This can be effected by raising the reservoir, and is necessary to make room for the water displaced by the gas subsequently evolved

[2] In case the temperature of the room is greatly different from 15 5° C (= 60° F) the measuring tube should be immersed in water at that temperature, but for ordinary purposes, and under ordinary conditions, this precaution is unnecessary

is given off, so that, to obtain the true amount of urea, the result indicated by Gerrard's apparatus should be multiplied by $92 - 99 = 0\ 93$

In the absence of any specially constructed apparatus for the estimation of urea, a suitable arrangement can be readily extemporised   Thus, if preferred, the nitrogen may be simply collected in a graduated tube filled with and standing over water, but it is more convenient to employ a graduated tube open at the upper end, which is drawn out so as to admit of being readily connected with the india-rubber tube attached to the evolution flask   An inverted Mohr's burette, of a capacity of 50 c c , immersed in a cylinder of water, answers very well   As long ago as 1877, A D u p r é (*Jour Chem Soc* , xxxi 534) recommended a tube of this kind, but furnished with a side-tube to which a clip was attached, which arrangement afforded some facilities in manipulating   These devices have all been superseded by the *nitrometer*, the employment of which for the purpose was first proposed by the a u t h o r (*Jour. Soc Chem Ind* , iv 179) [1]

Fig 11 shows the improved form of Lunge's nitrometer, furnished with a three-way tap, which enables communication between the nitrometer-tube and either the evolution-flask or the external air to be made at will, and materially facilitates the manipulation The method of working is almost the same as with Gerrard's form of apparatus   The hypobromite solution is placed in the flask, the tube charged with the sample of urine is carefully introduced, the stopper firmly adjusted, and the india-rubber leading-tube

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[1] D u p r e ' s apparatus appears to have been the first ureometer in which the principle of the nitrometer was adopted   The same principle was utilised in Gerrard's arrangement, the description of which was published a few months before the appearance of the a u t h o r ' s paper on " New and little known applications of the Nitrometer " , but the apparatus had been used and sketched by the a u t h o r some years previously

attached to the nose of the three-way tap The tap
is then turned, so as to allow the nitrometer-tube to
communicate with the external
air, and the open reservoir-tube
raised until the liquid in the
graduated tube just rises to the
zero-point, whereon the tap is
closed The tap is then turned
so as to connect the flask with
the nitrometer-tube, while cut-
ting off the external air The
urine is then allowed to come
gradually into contact with the
reagent in the manner already
described When the reaction is
complete, the liquid in the reser-
voir-tube is brought to the level
of that in the nitrometer, and
the volume of gas read off

In the absence of a Lunge's
nitrometer, the simpler form of

Fig 11

appaiatus devised by the author for assaying spirit
of nitrous ether (*Pharm Jour*, [3], xv 673), and
known as "Allen's nitrometer," will answer every
purpose The arrangement is shown in fig 12, but
there is no absolute necessity for the T-tube and spring
clip , though in their absence it is difficult to adjust
the level of the liquid in the nitrometer-tube to the
zero-point, and it becomes necessary to observe the
position when the water in the open and graduated
tubes stand at the same level

In the modified form of apparatus shown on page
130, a separating funnel is substituted for the sample
tube This arrangement allows the urine and reagent
to be brought together with any desired rapidity, or the
bromine to be added to the soda and urine previously

K
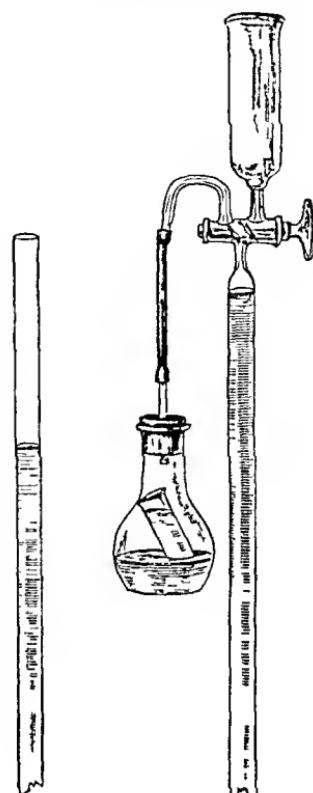
mixed together in the flask [1]   These and other variations in manipulation are very valuable in researches,
and in certain special applications of the nitrometer

For all ordinary purposes, it is convenient and
sufficiently accurate to fill the nitrometer with water,
but mercury is sometimes used in research-work

Another mode of using the nitrometer, and one
which does not necessitate the use of an instrument
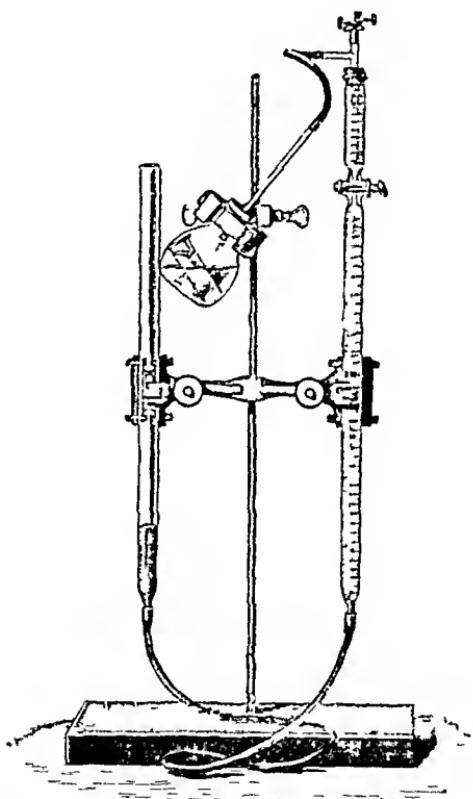
Fig 12

provided with a three-way tap or equivalent arrangement, has also been described by the a u t h o r (loc
cit)   For this purpose the nitrometer is filled to the

<hr>

[1] This mode of operating appears to be a valuable modification of the
ordinary plan, since a sensibly more complete evolution of the urcal nitrogen
is obtained, as was proved by experiments made in the author's laboratory by
A R Tankard   Twenty five c c of a 40 per cent solution of caustic soda is
placed in the flask, and 5 c c of the sample of urine added   The cork is then
adjusted and a solution of bromine in aqueous bromide of potassium (20 per
cent solution) added gradually from the tapped separator   In each case
the evolution of nitrogen occurred very promptly

tap with strong brine, and the tap closed   Five c c of the sample of urine [1] should then be placed in the cup by means of a pipette, and allowed to flow into the tube by opening the tap cautiously   The last traces are rinsed in by a few drops of water   Ten c c of the hypobromite solution, previously diluted with an equal measure of water, is next added through the tap   The greater part of the nitrogen is liberated as soon as the reagent comes into contact with the urine   When the reaction has somewhat abated, a clip is placed on the india-rubber connecting-tube, and the nitrometer-tube vigorously shaken   When the reaction is completed, the clip may be removed, the liquids in the nitrometer- and reservoir-tubes brought to the same level, and the volume of gas observed   If the reading be rendered difficult from the formation of a persistent froth, this may be instantly destroyed by introducing a few drops of alcohol through the tap

Instead of measuring the volume of gas evolved, it is sometimes convenient to measure the water displaced by it   This can be effected very simply by an arrangement devised by E R. S q u i b b (*Ephemeris*, ii 449)   A still simpler device, and one which can be readily extemporised, has been devised by the author   It consists simply of the apparatus shown in fig 3, page 47, with the addition of a test-tube to contain the sample of urine   One fluid ounce of the hypobromite mixture is placed in the feeding-bottle, and in the test-tube 2 fluid drachms (= 7 1 c c) of the sample of urine, together with a little simple syrup   The test-tube is placed inside the feeding-bottle, the stopper adjusted, and the contents of the tube allowed to mix with the hypobromite solution, just as when operating with the forms of apparatus

[1] If the volume of gas evolved exceed 40 c c , a smaller measure of urine should be employed

already described   The displaced water may be conveniently collected in a 2-oz measure   Operating with $\frac{1}{4}$ oz of urine, as prescribed above, each fluid ounce of water displaced represents 7 grains of urea per fluid ounce of the sample   Hence, if the measure collected were $1\frac{3}{4}$ oz ($= 14$ drachms), the urine contained $12\frac{1}{4}$ grains per fluid ounce   The percentage of urea may be found by dividing $12\frac{1}{4}$ by 4 375, or multiplying it by 0 2286, which in the example taken would give 2 80 per cent

In interpreting the results obtained by any of the modifications of the hypobromite method of estimating urea, it is unnecessary for ordinary purposes to take into account the barometric pressure, tension of aqueous vapour, or temperature at the time of making the experiment   By a fortunate coincidence, the increase in the volume of the gas, due to its being measured in a wet condition and at a temperature of 18° C ($= 65\ 4°$ F), almost exactly compensates for the $7\frac{1}{2}$ or 8 per cent of the total nitrogen ordinarily suppressed in the reaction   Thus R u s s e l l and W e s t (*Jour Chem Soc* , xxvii 749) found that 0·100 gramme of urea gave off 37 1 c c of moist nitrogen at a temperature of 65° F ($= 18\ 3°$ C) This, if dry, and at the standard pressure and temperature, would have measured 34 05 c c , whereas the *total* nitrogen contained in the same amount of urea would measure 37 2 c c under the standard conditions, or nearly the volume actually obtained at the ordinary temperature   Hence 37 1 c c of gas, measured under the ordinary conditions of experiment, may be taken to represent 0 1 gramme of urea   Thus, if the cubic centimetres of gas evolved be multiplied by $\dfrac{1000}{371} = 2\ 7$ (or, more exactly, 2 696) the product will be the number of milligrammes of urea in the volume of

urine employed , or, if 5 c c of urine were taken for the experiment, the volume of gas evolved, multiplied by 0 054 (or, more exactly, 0 0539) will be the grammes of urea (so-called " percentage ") in 100 c c of the urine   The percentage thus found, multiplied by 4 375, will be the number of grains of urea contained in 1 fluid ounce of the urine

If preferred, the above calculations can be combined, and the grains of urea per fluid ounce of the sample found at once by multiplying the measure of gas evolved from 5 c c of the sample by 0 236 (or, more exactly, 0 23585)

Instead of employing the foregoing factors, the content of urea per 100 measures and per fluid ounce can be found by reference to the following table, compiled by S. H e n r y S m i t h (*Pharm Jour* , [3], xxi 294) —

Volume of Nitrogen in c c evolved from 5 c c of Urine	Urea		Volume of Nitrogen in c c evolved from 5 c c of Urine	Urea	
	Per cent	Grains per oz		Per cent	Grains per oz
1	053	231	29	1 56	6 82
2	107	468	30	1 61	7 04
3	161	704	31	1 67	7 30
4	215	94	32	1 72	7 52
5	269	1 17	33	1 77	7 74
6	323	1 41	34	1 83	8 00
7	377	1 64	35	1 88	8 22
8	431	1 88	36	1 94	8 48
9	485	2 12	37	1 99	8 70
10	539	2 35	38	2 04	8 92
11	592	2 59	39	2 10	9 18
12	646	2 82	40	2 15	9 40
13	700	3 06	41	2 21	9 66
14	754	3 29	42	2 26	9 88
15	808	3 53	43	2 31	10 10
16	886	3 87	44	2 37	10 36
17	916	4 00	45	2 42	10 58
18	975	4 26	46	2 47	10 80
19	1 02	4 46	47	2 53	11 06
20	1 07	4 68	48	2 58	11 28
21	1 13	4 84	49	2 64	11 55
22	1 18	5 16	50	2 69	11 76
23	1 24	5 42	51	2 74	11 98
24	1 29	5 64	52	2 8	12 25
25	1 34	5 86	53	2 85	12 46
26	1 40	6 12	54	2 91	12 73
27	1 45	6 34	55	2 96	12 95
28	1 50	6 56	56	3 01	13 16

It must be borne in mind that the foregoing factors and table are intended for use with non-saccharine urine   As already stated, the evolution of the nitrogen of urea is more complete in the presence of sugar than in its absence, and hence the results yielded when diabetic urine is treated with hypobromite should be multiplied by the factor 0 93 to obtain more correct results. An alternative plan is to add some simple syrup or honey (from 3 to 5 c c) to the urine in the sample-tube   Under these circumstances, 40 c c. of nitrogen (against 37 1 c c in the absence of sugar) are yielded by 0 1 gramme of urea   Hence if 5 c c of urine be the volume employed, each c c of gas evolved represents 0 05 per cent of urea in the sample   Thus, if the nitrogen measure 26 4 c.c , the sample contains 26 4 × 0 05 = 1 32 per cent of urea

The author's experience of the hypobromite method of estimating urea, under a great variety of conditions, and with the utmost care to obtain accurate results, leads him to the conclusion that the process is by no means so accurate, nor even so constant in its indications, as is commonly supposed   But it is undoubtedly a method of great practical utility, and one which is capable of doing excellent service in the hands of intelligent operators   Its readiness of application, and the great rapidity with which fair approximations can be obtained, specially adapt it to the requirements of the physician, who might employ it with advantage far more frequently than is the case at present

INFLUENCE OF VARYING CONDITIONS ON THE EXCRETION OF UREA

The amount of urea excreted varies considerably with the diet, being increased by nitrogenous foods The weight of urea excreted per diem by an adult man on mixed diet ranges from 25 to 40 grammes,

the average being about 33 grammes (= 500 grains)
On a diet poor in proteids the excretion of urea may
fall to 15 to 20 grammes, while on a flesh diet the
daily output may rise to 100 grammes   The propor-
tion of urea in human urine averages about 2 per cent ,
but dog's urine is stated to contain 10 per cent

A large excretion of urea, if long continued, points to
increased tissue-metabolism or to surplus nitrogenous
ingesta, but a temporary increase may be simply due
to increased urination   Similarly, diminished excre-
tion of urea may be due to diminished metabolism or
to retention of urea in the system (as in uræmia).

A great number of observations have been recorded
of the influence of drugs, diseases, and other conditions
on the proportion of urea excreted   The results have
been classified by W D Halliburton (*Chemical
Physiology and Pathology*, 1891) as follow —

An increased excretion of urea occurs —

1   After administration of dilute sulphuric acid,
potassium chloride, ammonium salts (especially with
food), small doses of phosphorus, arsenic, antimony,
morphine, codeine, or large doses of quinine

2   After poisoning by phosphorus or arsenic.

3   From application of cold to the skin , after hot
baths , from increase of oxygen inhaled , from exces-
sive muscular work

4   In diseases, as at the commencement of acute
febrile diseases, up to the acme of the fever , during the
paroxysms of intermittent fever (ague), in diabetes.

A decreased excretion of urea occurs —

1   After administration of small doses of quinine

2   During the sinking of the fever in acute febrile
diseases , in most chronic and debilitating diseases
(anæmia, syphilis, phthisis, dropsical affections, &c) ,
towards the fatal termination of most diseases (5 to 6
grammes daily) , in uræmia (when the excretion may

entirely cease), in diabetic coma , and in all degenerative changes of the liver, especially in acute yellow atrophy

Creatinine. Methyl-glycocyamidine

$$C_4H_7N_3O, \text{ or, } NH \quad C \left\{ \begin{array}{l} N(CH_3) \, CH_2 \\ NH.CO \end{array} \right\}$$
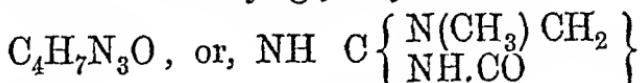
Creatinine is an anhydride of c r e a t i n e, $C_4H_9N_3O_2$, and is produced from the latter body with great facility [1] Creatinine occurs constantly in normal human urine, the amount varying, according to V o i t, from 0 5 to 4 9 grammes per diem, according to the quantity of proteids eaten. The proportion is not diminished by fasting, but is said to be increased in typhus, intermittent fever, pneumonia and tetanus, and diminished in convalescence from acute diseases, anæmia, chlorosis, paralysis and phthisis Creatinine has been found in sweat and the muscles of fishes, and G S J o h n s o n has isolated creatinine, or a modification of it, from the flesh of a healthy cow

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[1] CREATINE , Methyl-glycocyamine , or Methyl guanidine acetic acid

$$C_4H_9N_3O_2, \text{ or, } NH \quad C \left\{ \begin{array}{l} N(CH_3) \, CH_2 \, COOH \\ NH_2 \end{array} \right\}$$
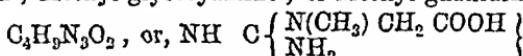
Creatine is a constant constituent of muscle substance, to the extent of 0 2 to 0 3 per cent of its weight, and is most conveniently prepared from Liebig's extract of meat Creatine has also been found in nerve tissue, and probably occurs in minute traces in urine and other animal fluids But its isolation from these does not prove its pre existence therein, since it is very easily formed by the dehydration of creatinine, into which body, on the other hand, creatine is very readily changed Thus, on evaporating a solution of creatine with the calculated amount of dilute sulphuric acid, it yields a residue of creatinine sulphate A similar change occurs very readily on boiling creatine with dilute hydrochloric acid, and the result-

Fig 13 —CREATINE (after Frey)

ant creatinine can be readily recognised by conversion into the zinc salt

Creatine crystallises in transparent rhombic prisms (fig 13) containing water It is sparingly soluble in cold water to form a slightly bitter solution, neutral to litmus It reduces alkaline picric acid solution gradually in the cold, but immediately on boiling
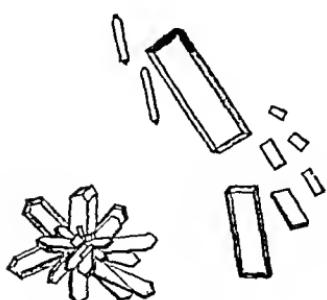
Creatinine may be isolated from human urine by Liebig's process, which consists in exactly neutral-ising the liquid with milk of lime, and adding calcium chloride as long as calcium phosphate continues to be precipitated The filtrate, which should be neutral or very faintly acid, is evapo-rated to a small bulk, and the crystals of common salt, &c, removed Thirty-two parts of the mother-liquor are treated with one part

Fig 11 —CREATININE (after Frey)

of zinc chloride in very concentrated solution, and the whole left for several days The creatinine-zinc chloride which separates in nodules is washed with a little cold water, and then with alcohol It is then boiled with recently precipitated lead hydroxide, the filtrate evaporated, and the residue digested with absolute alcohol, which dissolves the creatinine leav-ing any creatine insoluble

G Stillingfleet Johnson prepares creat-inine by a modification of Maly's process [1] He treats a large volume of urine with 5 per cent of its volume of a saturated aqueous solution of sodium acetate and 25 per cent of saturated mercuric chloride solution The precipitate which forms is filtered off immediately, and the filtrate left at rest for forty-eight hours The creatinine separates in microscopic spherical masses of the mercuric chloride compound, which is filtered off, washed with cold water, and decomposed by sulphur-

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[1] Maly (*Ann Chem Pharm*, clix 279) recommends a preliminary con-centration of the urine by heat, and precipitation by basic acetate of lead before adding mercuric chloride Johnson's modification is a marked improve-ment on this, as it gives a purer product and wholly avoids the use of heat, which is an essential condition if it be desired to obtain unchanged creatinine

etted hydrogen.   The filtered liquid is decolorised with purified animal charcoal, and concentrated by spontaneous evaporation over sulphuric acid, when creatinine hydrochloride separates in brownish-yellow prisms   The crystals are redissolved in 15 parts of cold water, and the solution treated with an excess of lead hydroxide, prepared by precipitating a solution of the acetate with ammonia   The mixture is well stirred for twenty minutes and then filtered   The filtrate is free from lead and chlorine, and on evaporation over sulphuric acid in a vacuum yields free creatinine in the form of efflorescent crystals containing $C_4H_7N_3O + 2H_2O$ If this creatinine be dissolved in hot water the crystals obtained on spontaneous evaporation are anhydrous tables, which, according to G S Johnson, are not chemically identical with the efflorescent form, but reconvertible into it by modifying the process of solution   The following table shows the results obtained by Johnson by the recrystallisation of "artificial" urinary creatinine (*Proc Royal Soc*, xliii 526), reproduced from creatine which had been prepared by boiling urinary creatinine with water —

Nature of Crystals	Solvent	Evaporation	Product
Effloresced $K\alpha$ Tabular creatinine α	Water at 60° C	In vacuo	Tabular $K\beta$
	Water at 60° C	In vacuo	Efflorescent $K\beta$
Effloresced $K\alpha$ Tabular $K\beta$ (anhydrous)	Water at 100° C	In vacuo	Tabular $K\alpha$ (anhydrous)
	Cold water	In vacuo	Efflorescent $K\alpha$
Efflorescent $K\beta$	Cold water	In vacuo	Efflorescent K

Creatinine as ordinarily obtained crystallises in oblique rhombic prisms and stellate forms (fig 14) It dissolves in about 12 parts of cold water, and is also soluble in alcohol, but almost insoluble in ether

The aqueous solution of creatinine is, according to some observers, neutral, but according to others

alkaline[1] The solution readily undergoes change with formation of c r e a t i n e, especially if ammonia, oxide of lead, or other base be present By prolonged boiling with caustic alkali, creatinine is completely decomposed

By boiling with baryta-water, creatinine is hydrolysed to a m m o n i a and m e t h y l-h y d a n t o i n,

$$C(NH) \left\{ \begin{array}{l} N(CH)_3\ CH_2 \\ NH\text{——}CO \end{array} \right\} + H_2O = NH_3 + CO \left\{ \begin{array}{l} N(CH_3)\ CH_2 \\ NH\text{——}CO \end{array} \right\}.$$
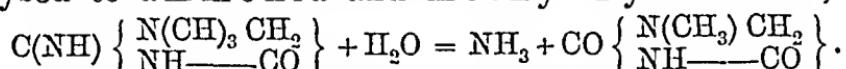
Boiled with water and mercuric oxide, it gives methylguanidine

Creatinine yields a series of crystallisable salts The *hydrochloride*, BHCl, crystallises in prisms from alcohol or in laminæ from water It unites with zinc chloride to form the double salt $ZnCl_2,2BHCl$ This is very soluble in water and alcohol, and must not be mistaken for the compound $ZnCl_2,2C_4H_7N_3O$, which is one of the most characteristic salts of creatinine *Creatinine-zinc chloride* is obtained by mixing concentrated aqueous or alcoholic solutions of zinc chloride and creatinine, or by adding sodium acetate to the solution of the double hydrochloride It forms oblique rhombic prisms or small needles, which have a tendency to form rosettes or warty concretions The crystals are soluble in about 54 parts of cold or 27 of boiling water They are insoluble in absolute alcohol, and require 9217 parts of alcohol of 98 per cent, or 5734 of alcohol of 87 per cent

Mercuric chloride gives a white, curdy precipitate in strong solutions of creatinine, but the separation is not perfect unless sodium acetate be added, or mercuric acetate substituted for mercuric chloride On allowing such a mixture to stand at the ordinary temperature, the compound is gradually deposited in

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[1] A strongly alkaline sample leaves an alkaline ash on ignition, proving the presence of mineral impurity S a l k o w s k i finds creatinine quite free from alkaline reaction, but it liberates ammonia from ammoniacal salts on boiling

microscopic spherules   This reaction is applied by G
S Johnson to the isolation of creatinine from urine
(*Proc Royal Soc*, 1888, xliii 507)   The compound
is almost insoluble in cold water, and is decomposed
with partial reduction of the mercury by hot water
It is readily soluble in dilute hydrochloric acid, but is
nearly insoluble in acetic acid   Johnson attributes
to the spherical mercury compound the formula
$C_{16}H_{28}N_{12}Hg_7Cl_{10}$, and suggests the following constitu-
tion —$4(C_4H_5Hg''N_3O,HCl),3HgCl_2 + 2H_2O$ Experi-
ments on specimens of the spherical salt prepared
in the author's laboratory, both from urine and from
pure creatinine prepared by Johnson's process (page
154), do not fully confirm this formula.   The propor-
tion of chlorine, in particular, varies materially, a fact
which points to the presence of HgO, and possibly of
Hg_2Cl_2, in some preparations

From a concentrated solution of creatinine, silver
nitrate precipitates crystals of the compound,
$C_4H_7N_3O,AgNO_3$ Mercuric nitrate does not precipi-
tate a dilute solution of creatinine till excess of sodium
carbonate is added, when $B_2Hg(NO_3)_2,HgO$ is thrown
down as a crystalline precipitate

Creatinine possesses marked reducing properties
The mercury of the spherical salt above described is
reduced, even in the cold, partly to the mercurous
state and partly to metal on adding caustic alkali.
Contact with boiling water produces a similar change

Creatinine reduces Fehling's solution on boiling, the
blue liquid changing to yellow, but no cuprous oxide
separates   Creatinine appears also to prevent the
separation of a precipitate when glucose is present, and
hence exerts an interfering action on the application
of Fehling's solution to the detection of dextrose in
urine (compare page 65)   Pavy's solution is reduced
by creatinine without precipitation, and the reagent

may be used for its determination   According to G S Johnson, the reducing power of creatinine obtained direct from urine is greater than that of the base prepared from creatine (compare page 154)   Thus he finds 12 grammes of tabular creatinine-α from urine to have a cupric oxide reducing power equivalent to 12 grammes of glucose , that is two molecules of this creatinine equal one of glucose in reducing power , against $2\frac{1}{2}$ molecules required of creatinine prepared from creatine

Phosphomolybdic and phosphotungstic acids produce micro-crystalline precipitates in solutions of creatinine acidulated with nitric or hydrochloric acid   By treating the precipitates with baryta, free creatinine is obtained

If a concentrated solution of picric acid be added to normal human urine a small crystalline sediment is gradually formed   On separating this, and treating it with hot water, uric acid remains, while the greater part dissolves   The soluble portion is a double picrate of potassium and creatinine, which forms lemon-yellow needles or thin prisms, readily soluble in hot water, sparingly in cold alcohol, and almost insoluble in ether   With dog's urine the precipitate produced by picric acid contains little or no uric acid, and the kynurenic acid present is not precipitated

When a solution of picric acid is added to a solution of creatinine not more dilute than 1 in 3000, on adding a drop of dilute caustic alkali a deep red colour is produced, which is intensified by boiling the liquid   By this reaction the presence of creatinine can be recognised in the urine of man, dog, and rabbit   Acetone gives a similar but less intense colour   Glucose gives a similar reaction on heating   It is evident that the behaviour of creatinine with picric acid gravely affects the value of that reagent as a test for small quantities of sugar in urine

T Weyl (*Berichte*, 1878, page 228) has pointed out that if a few drops of very dilute solution of sodium nitroprusside be added to a solution of creatinine, and dilute caustic soda then added drop by drop, a fine ruby-red colour will be produced, which in a few minutes changes to an intense straw-yellow   If the liquid be now acidulated with acetic acid and warmed, it turns greenish and prussian blue separates   G u a r - e s c h i recommends that 10 per cent solutions of nitroprusside and caustic soda should be used   K r u - g e n b e i g states that the reaction is best obtained by first adding caustic soda, and then a few drops of a concentrated solution of the nitroprusside.   S a l k o w - s k i confirms this   The reaction is very delicate, and can be obtained with a solution containing 0 03 per cent of pure creatinine, or with urine containing 0 066 per cent   In applying the test to urine the absence of acetone should be insured by distilling off a portion, since that body gives a ruby-red colour with Weyl's test, though no blue colour can be obtained on acidu- lating, acetic acid merely restoring the yellow colour to red   According to G u a r e s c h i, a red colour is also yielded by hydantoin, methyl-hydantoin, and other compounds containing the group N CH$_2$ CO N Creatine gives no reaction with Weyl's test unless the liquid be first boiled with a dilute acid, so as to convert it into creatinine   In this manner, Weyl demonstrated the presence of creatine in milk (*Berichte*, xi 2175)

The determination of creatinine in urine is usually based on its isolation as creatinine-zinc chloride, which process is preferred by N e u b a u e r   P. G r o c c o (*Chem Centr*, 1887, page 17) has described the follow- ing modification of this method of isolating creatinine from urine   The urine of twenty-four hours, kept acid by acetic acid, and preserved in ice if possible, is treated with milk of lime till only faintly acid, calcium

chloride added and the liquid evaporated, the reaction being maintained neutral or faintly acid by cautious addition of acetic acid   The residue is extracted with alcohol containing a little sodium acetate, and the filtered liquid treated with alcoholic zinc chloride and a little acetic acid, avoiding excess   If necessary, the alcoholic extract is decolorised with animal charcoal before adding the zinc reagent, and it should be cooled with ice   The purity of the precipitated creatinine-zinc chloride should be proved by a microscopic examination, with a high power, to make certain of its freedom from sodium chloride   It should be completely soluble in hot water   (Compare page 155)

E S a l k o w s k i operates in a very similar manner, but uses a smaller quantity of urine   He directs that 240 c c should be rendered alkaline by the cautious addition of milk of lime, and precipitated by calcium chloride   The volume is made up to 300 c c , and the liquid filtered after ten minutes   250 c c of the filtrate, representing 200 of urine, which must be feebly alkaline, is evaporated to about 20 c c , and an equal measure of absolute alcohol added   This is subsequently diluted to 100 c c with alcohol, allowed to stand twenty-four hours, and filtered   To 80 c c of the filtrate zinc chloride is added, and the creatinine-zinc chloride collected after twenty-four hours

Instead of weighing the compound of creatinine with zinc chloride, the contained creatinine may be deduced from the amount of ammonia produced on decomposing it with boiling concentrated sulphuric acid For this purpose the precipitate should be dissolved in the minimum quantity of sulphuric acid, previously diluted with an equal measure of water, and the solution treated as described on page 128

A method of isolating creatinine from urine, which, in the opinion of the author, is preferable to the zinc

process, is that based on its precipitation as the spherical mercuric compound (described on page 156), which, according to G S Johnson, contains about 20 per cent of creatinine   Instead of weighing the precipitate, it may be washed with cold water, and decomposed by treatment with strong sulphuric acid as in Kjeldahl's process [1] described on page 127, the creatinine being deduced from the amount of ammonia obtained   This plan, in the opinion of the a u t h o r, is preferable to weighing the mercury salt

Attempts to determine creatinine by treating the zinc or mercury compound with alkaline hypobromite solution, as employed for the estimation of urea, have failed to give the author satisfactory results, the proportion of the total nitrogen evolved on treatment with the hypobromite being apparently dependent on conditions not yet mastered   Hence the method, which at first appeared very promising, cannot at present be recommended

Uric Acid.   Lithic Acid $C_5H_4N_4O_3$

Uric acid is one of the most constant and characteristic products of the metabolism of the animal organism   In its formation the nuclein of the cell-nuclei is specially concerned   It exists normally as urate of sodium in human urine, the proportion present being greatly increased in cases of gout and rheumatism, when the urine contains large quantities of acid urate of sodium [2]   Traces of uric acid exist normally in the

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[1] Kjeldahl's original method must be employed, as the modified process described on page 128 is not applicable in presence of mercury compounds

[2] The amount of uric acid commonly excreted by an adult is usually stated to be about 8 grains (0 5 gramme) in the twenty four hours, but the results of the more modern methods of determination lead to the conclusion that the diurnal quantity eliminated under normal conditions is from 20 to 30 grains (1 3 to 2 0 grammes)   The deposition of urates from urine on cooling does not prove their presence in excessive amount

During recent years, H a i g and other observers have shown that uric acid

brain, lungs, liver, and spleen, and uric acid is also found in the saliva, gastric juice, sweat, &c   The merest trace exists normally in blood, but in cases of albuminuria, and especially of gout, the proportion becomes very appreciable   The so-called "chalk-stones," and other gouty concretions, commonly consist of the sparingly soluble acid sodium urate, while the buff-coloured sediment which frequently separates from human urine usually consists of the quadri-urate of sodium or ammonium   Acid urate of ammonium constitutes the greater part of the urinary excrement of birds ("guano"), while that of serpents and other terrestrial reptiles contains it in a still purer form   On the other hand, uric acid is nearly absent from the urine of herbivorous mammals, being replaced therein by hippuric acid

The synthesis of uric acid has been effected[1] by H o r b a c z e w e s k i by heating glycocine with ten times its weight of urea to about 230° C —

$$C_2H_5NO_2 + 3CH_4N_2O = C_5H_4N_4O_3 + 2H_2O + 3H_3N$$
Glycocine   Urea   Uric acid   Water   Ammonia

has, in all probability, a much wider bearing in pathology than had previously been supposed   Its connection with gout, rheumatism, and stone has long been recognised, though its relation to these complaints has been much misunderstood   Its relation to other affections (e g , according to Haig is a casual factor in migraine) is still more obscure

[1] Another interesting synthesis of uric acid has been effected by B e h r e n d and R o o s e n (Berichte, xxi 999) by the reaction of etho acetic ether and urea   From this synthesis the following constitutional formula, first proposed by M e d i c u s, has been assigned to uric acid —

$$CO \begin{cases} NH-CO \\ \quad\quad C-NH \\ NH- C -NH \end{cases} CO$$
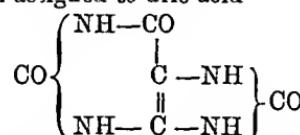
This formula shows that uric acid contains the residues of two molecules of urea, and explains the fact that the decompositions of uric acid almost invariably yield either a molecule of urea or some derivative of urea, together with a second body which can by further treatment be converted into urea   Many of the decomposition products of uric acid can indeed be prepared directly from urea   In view of the close relationship existing between urea and uric acid, it is not surprising that the foods which, in the mammal, cause an increased excretion of urea, in birds are converted into uric acid

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Conversely, when uric acid is heated under pressure to 170° with hydriodic acid, it yields glycocine, ammonia, and carbon dioxide

Uric acid differs by an atom of oxygen from x a n t h i n e, a feeble base of wide occurrence in both the animal and vegetable kingdoms, and the physiological and pathological relations of which are but little understood [1]

[1] XANTHINE, $C_5H_4N_4O_2$, is the typical member of a series of feebly basic bodies closely related to uric acid and to each other   Some of these compounds (e g , xanthine, hypoxanthine, guanine, carnine) occur in small quantity in normal urine and animal organs and tissues, and are normal products of the degradation of proteids   Other members of the group (e g , caffeine, theobromine, theophylline, and xanthine itself) occur in plants

Hypoxanthine ($C_5H_4N_4O$), *xanthine* ($C_5H_4N_4O_2$), and *uric acid* ($C_5H_4N_4O_3$) all occur in normal urine   They differ from each other only by an atom of oxygen, but, notwithstanding this close relationship, they do not seem to be convertible, as has been alleged   *Heteroxanthine* ($C_6H_6N_4O_2$) and *paraxanthine* ($C_7H_8N_4O_2$) also occur in normal urine, and the former is said to be present in larger amount in the urine of anæmic persons

The vegetable bases *theobromine* and *theophylline* are d i m e t h y l - x a n t h i n e s, $C_5H_2(CH_3)_2N_4O_2$, while *caffeine* has the constitution of a t r i m e t h y l - x a n t h i n e, $C_5H(CH_3)_3N_4O_2$

Guanine, $C_5H_5N_5O$, a body abundant in Peruvian guano, has the constitution of an i m i d o - x a n t h i n e, while *adenine*, $C_5H_5N_5$ (originally isolated from the pancreas) bears the same relationship to hypoxanthine

Xanthine was originally discovered (M a r c e t, 1819) in a urinary calculus   It has been found in guano, and is a normal constituent of urine, especially during the use of sulphur baths, and is present in minute quantities in various parts of the system   It has also been found in tea, lupines, malt seedlings, yeast, &c , and has been produced synthetically

In their chemical and physical characters, the xanthine bases present a close resemblance to uric acid   They have but a feeble affinity for acids, and their salts are mostly decomposed by water   Some of them (including xanthine itself) exercise an acid function in addition, and unite with bases   They are mostly very slightly soluble in cold water, and, except caffeine and theobromine, insoluble in alcohol, ether, or chloroform   They all yield white precipitates with phosphomolybdic acid, mercuric chloride, and ammoniacal lead acetate , and guanine and adenine are very perfectly precipitated by picric acid

A general reaction of the xanthine bases is their precipitation from ammoniacal solutions by ammonio nitrate of silver, as a gelatinous compound of the base with argentic oxide   On treating the precipitate with dilute nitric acid, crystalline compounds of the bases with silver nitrate are obtained, the xanthine compound containing $C_5H_4N_4O_2,AgNO_3$ The different solubility of these compounds in water and nitric acid affords a means of distinguishing and separating the xanthine bases from each other

The xanthine derivatives (except caffeine and theobromine) are precipitated

As commonly obtained, uric acid is said to be anhydrous,[1] and forms small crystalline scales which are very apt to appropriate colouring matter   The microscopic

by cupric acetate, especially on heating   The precipitates are the cuprous salts of the bodies, that yielded by xanthine containing $Cu_2O,C_5H_4N_4O_2$ They may be produced by treating the neutral solution of the body with a mixture of cupric sulphate and sodium sulphite or thiosulphate, or by mixing the ammoniacal solution with Fehling's solution, heating to boiling, and gradually adding a solution of dextrose   Instead of cuprous oxide separating in the free state, it combines with the xanthine derivative to form a white insoluble compound   Hence it is evident that the presence of xanthine and its allies, including uric acid, will prevent the detection of sugar in urine by Fehling's test to an extent dependent on the amount of the interfering body present   The fact is of considerable practical importance when small quantities of sugar are to be sought for, and therefore, in such case, Fehling's test should be applied in the modified manner described on page 62   If, instead of using dextrose as a reducing agent, the mixture of a xanthine base with Fehling's solution be treated with hydroxylamine hydrochloride reduction of the copper to the cuprous state will occur in the strongly alkaline solution and at the ordinary temperature   P B a l k e (*Jour Pract Chem* , [2], xlvii 537) employs the foregoing reaction for the determination of the xanthine derivatives, and for their isolation from flesh, malt, &c

The proportion of xanthine derivatives (other than uric acid) ordinarily present in urine is extremely small, but there is reason to believe that, under circumstances not fully understood, their amount is much increased and may then be of pathological importance   For the extraction of xanthine from urine, a large quantity (5 to 10 gallons) should be treated by instalments of about 1 quart at a time with neutral lead acetate in powder, as long as a precipitate is produced   The liquid is filtered and sodium sulphate added as long as lead sulphate is thrown down, the liquid poured off or filtered from the precipitate, copper sulphate added, and the liquid boiled   The precipitate, which contains the xanthine derivatives as cuprous salts, is filtered off, washed, dissolved in dilute nitric acid, and excess of ammonia added, followed by silver nitrate   The precipitate, consisting of the argentic oxide compounds of xanthine, &c , is separated, suspended in hot ammoniacal water, and decomposed by sulphuretted hydrogen, the resultant silver sulphide filtered off, and the filtrate concentrated till the xanthine crystallises out   If, instead of decomposing the silver precipitate with sulphuretted hydrogen, it be boiled with nitric acid of 1 10 specific gravity, the silver nitrate compounds of hypoxanthine and adenine will crystallise out immediately on cooling, while those of xanthine, paraxanthine, and heteroxanthine will remain in solution, and may be recovered as the silver-oxide compounds by rendering the filtrate ammoniacal

[1] When slowly deposited from dilute solutions, uric acid sometimes separates in large crystals containing $C_5H_4N_4O_3+2H_2O$ It is very probable that some of the ordinary forms of uric acid are hydrated   Thus Dr James Edmunds finds that all crystals of uric acid, obtained by addition of hydrochloric acid to cold filtered urine, effloresce and break up on heating to 200° F   or on exposure in a desiccator at 60° F over sulphuric acid for twenty-four hours   Preparations made by Dr Edmunds show this in a marked manner

appearance of uric acid is very variable, dumb-bell, whetstone, and lozenge-like forms being among the most characteristic (fig 16, page 167) When precipitated from urine by adding hydrochloric acid, it commonly forms small, transparent, rhombic tablets, with a few elliptical and oblong plates, but the forms assumed greatly depend on the nature and amount of the pigments and other co-existing substances

When quite pure, uric acid forms a white crystalline powder without taste or smell, and of a specific gravity ranging from 1 855 to 1 893 On heating, it decomposes without melting, giving off hydrocyanic acid and carbon dioxide, yielding a sublimate containing cyanuric acid, ammonium cyanate and urea, and leaving a carbonaceous residue

Uric acid is nearly insoluble in water, requiring 15,000 parts of cold or 1800 of boiling water for its solution Blarez and Deniges (*Compt rend,* civ, 1847) find that 100 grammes of water at 0° C dissolve 2 0 milligrammes of uric acid, at 10°, 3 7, at 20° 6 0, and at 100°, 62 5 milligrammes of uric acid Uric acid dissolves in glycerin, but in alcohol and ether it is quite insoluble Uric acid is soluble in solutions of the borates, phosphates, carbonates, acetates, and lactates of potassium and sodium, but not in solutions of the corresponding ammonium salts

In strong sulphuric acid uric acid dissolves to form a crystallisable sulphate, which is decomposed by water, the uric acid being precipitated unchanged When strongly heated with concentrated sulphuric acid, uric acid is broken up, the nitrogen being ultimately wholly converted into ammonia (see page 127)

Hydrochloric acid has neither solvent nor chemical action on uric acid By the action of oxidising agents on uric acid a number of compounds of great theoretical interest are obtainable These form two dis-

tinct series   The first, represented by a l l o x a n, $C_4H_2N_2O_4$, are produced by acid oxidising agents, such as nitric acid   The second, of which a l l a n t o i n, $C_4H_6N_4O_3$, is the type, result from the oxidation of uric acid in alkaline or neutral solution

Hot dilute nitric acid converts uric acid into a l l o x a n t i n, $C_8H_4N_4O_7$, a body which is also produced by the action of reducing agents on alloxan   Chlorine and bromine convert uric acid at ordinary temperatures into u r e a and a l l o x a n   On heating, p a r a b a n i c and o x a l i c a c i d s are also produced   Hypobromites and hypochlorites cause the evolution of a portion of the n i t r o g e n of uric acid in a gaseous state, but the reaction does not appear to be sufficiently constant to serve as a method of determining uric acid

Uric acid suspended in pure water remains unchanged for a long time, but the addition of a very small quantity of decomposed urine causes its rapid and complete decomposition in hot weather, with formation of a m m o n i u m c a r b o n a t e and other bodies

DETECTION AND DETERMINATION OF URIC ACID

Uric acid is commonly separated in the free state by adding excess of hydrochloric acid to its solution   When separated from urine in this manner, it forms a coloured deposit which adheres to the sides of the glass [1]   The best mode of operating is described in the sequel

When isolated, uric acid is readily identified by its microscopic appearance, though the forms it assumes are very numerous   When deposited from urine or other impure solutions, dumb-bell, whetstone, and lozenge-like forms are among the most common and

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[1] A drop of fresh urine, mixed with hydrochloric acid, may be observed under the microscope to deposit uric acid crystals in the course of a few minutes

characteristic (fig 16, *b* and *c*)   A E Garrod has shown that the pigments of urine are especially concerned in modifying the forms assumed by the uric

acid, and that the presence of excess of one particular pigment will produce a corresponding definite variation in the form of the crystals   Dr J Edmunds has independently found that the forms assumed by uric acid greatly depend on the nature and amount of the co-existing substances When precipitated from a solution of a pure urate by addition of hydrochloric acid, uric acid generally forms minute transparent rhombic plates (fig 16, *a*)   Large crystals are obtainable much more readily from urine or other impure solutions than from pure urates

A highly characteristic and delicate reaction is that known as the "murexide test," which is based on the behaviour of uric acid on
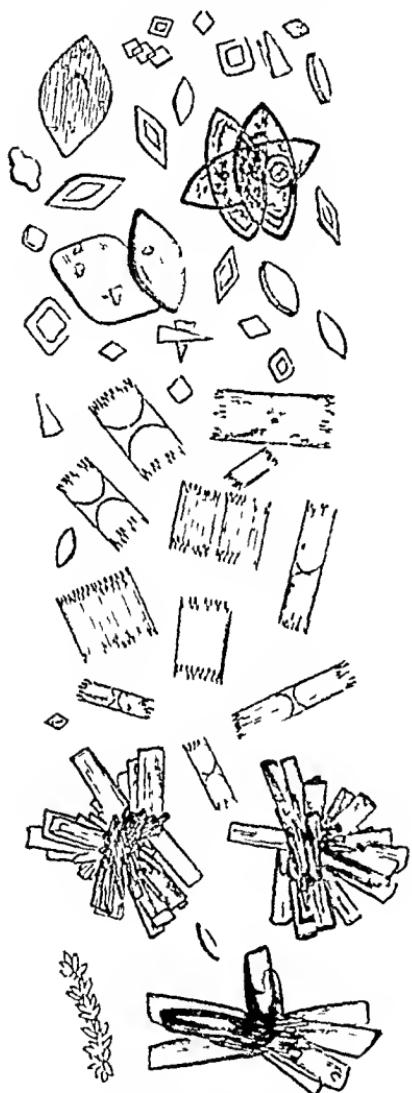
Fig 15 — CRYSTALS OF URIC ACID

oxidation   If uric acid, a urate, or even urine be treated with a few drops of strong nitric acid, and the liquid evaporated to dryness in porcelain at 100°, a yellowish or red residue will be obtained, which owes its colour to the formation of alloxantin, $C_8H_6N_4O_8$ On inverting the capsule over another containing ammonia, or otherwise subjecting the

residue to ammoniacal vapours, it acquires a magnificent purple colour, owing to the formation of murexide or a m m o n i u m p u r p u r a t e, $NH_4 C_8H_4N_5O_6$

On now adding caustic soda, the purple becomes changed to blue, the colour disappearing on warming   Somewhat · analogous reactions are given by caffeine, theobromine, guanine, and xanthine, but the differences do not allow of their confusion with uric acid

Fig 16 —CRYSTALS OF URIC ACID —*a*, From decomposition of urates , *b*, from human urine, *c*, dumb bell forms

The nitric acid prescribed in the above test may be advantageously replaced by bromine-water, or the material to be tested may be evaporated with a few drops of strong hydrochloric acid and a minute crystal of potassium chlorate

If uric acid be dissolved in a solution of sodium carbonate and a drop of the liquid placed on filter-paper previously moistened with silver nitrate, a yellow, brown or black spot will be produced, owing to the fact that silver carbonate is reduced by uric acid even at the ordinary temperature

On adding a little Fehling's solution to a solution of uric acid in caustic soda, a greyish precipitate is formed, said to consist of cuprous urate , but with excess of the reagent, and on application of heat, red cuprous oxide separates

Uric acid does not reduce a hot, alkaline solution of picric acid, which fact distinguishes it from creatinine, glucose, and other normal and occasional constituents of urine which do react with Fehling's solution

For the *determination* of uric acid in urine a common practice is to add hydrochloric acid to the

previously concentrated sample, allow the liquid to stand in the cold for a few hours, and filter from the precipitated uric acid, which is subsequently washed with cold water and rectified spirit, dried, and weighed. But under the most favourable circumstances, and however carefully the process be conducted, the separation of the uric acid is incomplete, and the results consequently below the truth

A preferable method of isolating the uric acid from urine is that based on the insolubility of the acid ammonium urate, $C_5H_3(NH_4)N_4O_3$, in a solution of ammonium chloride or sulphate   In the original process, which is due to A P F o k k e r (*Jour Chem Soc ,* xxviii 1293 , also S a l k o w s k i , *Fresenius' Zeitschrift,* xvi 371), only a limited amount of ammonium sulphate was used, and hence a considerable correction was necessary for the solubility of the acid urate , but F G H o p k i n s (*Chem News,* lxvi 106) has pointed out that by saturating the liquid with ammonium chloride no such correction is required, and the time necessary for complete precipitation is much reduced Hopkins prescribes the following procedure —

To 100 c c (or 4 fluid ounces) of urine, finely powdered ammonium chloride is added in excess,[1] about 30 grammes (1 oz) being necessary   When a small quantity of the salt remains undissolved, even after brisk stirring at intervals of a few minutes, saturation is sufficiently complete, even if complete solution occurs when the liquid recovers from the depression of temperature caused by the solution of the ammonium chloride   The solution is allowed to stand for two hours with occasional stirring, and is then passed

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[1] When it is intended subsequently to decompose the precipitate by hydrochloric acid and weigh the liberated uric acid, it is essential that the ammonium chloride used should dissolve to an absolutely clear solution in water, since the quantity employed is very large relatively, and any insoluble matter would seriously vitiate the estimation of uric acid

through a thin filter and washed twice with a saturated solution of ammonium chloride   When time is an object, the urine may be made alkaline with ammonia after saturation with ammonium chloride   The phosphates which are thus precipitated with the ammonium urate occasion no inconvenience, while precipitation is complete in ten minutes

When a normal acid urine is saturated with pure ammonium chloride, the precipitate of acid ammonium urate, after being washed with the cold, saturated solution of ammonium chloride, yields a mere trace of ash on ignition, showing that no mineral salts are carried down   Of the ordinary constituents of urine, uric acid, xanthine, and certain pigments appear to be the only bodies precipitated   *Xanthine* is thrown down still more completely from ammoniacal solutions, but it is left in solution when the ammonium urate is subsequently decomposed by hydrochloric acid   Hypoxanthine and creatinine are not precipitated by ammonium chloride   Certain *pigments* are thrown down, so that the precipitate is always more or less coloured   *Hæmato-porphorin*, in particular, is very perfectly precipitated, but remains in solution when the urate is subsequently decomposed by acid

The acid ammonium urate, isolated in the foregoing manner, admits of several alternative treatments, as follow —

1   When it is desired to determine the uric acid by weight, the precipitate is rinsed off the filter with a jet of hot water, and the liquid heated just to boiling with excess of dilute hydrochloric acid   The liquid is thoroughly cooled and allowed to stand for two hours   It is then filtered on to a smooth filter, and the crystals of uric acid washed twice with cold water, then with alcohol, till the washings are no longer acid, dried at 100° C (= 212° F), and weighed   To the weight of

uric acid thus obtained 0 001 gramme should be added for every 15 c c ($= \frac{1}{2}$ oz) of mother-liquor, the bulk of which need never exceed 30 c c , but no correction need be made for the insignificant trace of uric acid dissolved by the aqueous and alcoholic washings   The uric acid thus isolated is usually only slightly coloured and is practically pure   When derived from highly pigmented urines, the uric acid may retain so much colouring matter as to suggest the presence of an amount of impurity sufficient to vitiate the result   In such case, after washing the precipitate of acid ammonium urate off the filter, rectified spirit equal in bulk to the water present should be added, and, after adding hydrochloric acid, the beaker should be covered and heated for some time on the water-bath

Instead of weighing the uric acid isolated in the foregoing manner, it may, if preferred, be dissolved in a little hot solution of sodium carbonate, and the liquid treated by process 3 or 4.

2 The precipitate of acid ammonium urate having a perfectly definite composition, it may be titrated with standard alkali and an indicator giving no reaction with uric acid   Such an indicator exists in methyl-orange   ·The precipitate from 200 c c of urine is treated with a known measure, e.g , 20 c c, of deci-normal hydrochloric or sulphuric acid, the liquid boiled for some minutes, cooled, diluted to 200 c c, a few drops of a 1 per cent aqueous solution of methyl-orange added, and $\frac{1}{20}$-normal caustic soda ($= 2\ 0$ grammes of NaHO per litre) dropped in from a burette until the orange colour of the acid liquid becomes yellow, which change indicates the point of neutrality. The difference between the volume of acid employed and that of the alkali required to neutralise it represents the ammonia of the precipitate, uric acid having no action on methyl-orange   Each centimetre of $\frac{N}{20}$

solution of soda shows the presence of 0 0084 gramme of uric acid

3 An alternative plan, when the acid urate of ammonium is not very strongly coloured, is to rinse the precipitate off the filter with hot water, cool the solution, and dilute it with distilled water to 100 c c   Twenty c c of pure concentrated sulphuric acid is then added, so as to acidify the liquid and raise its temperature to about 60° C (= 140° F.), and then a standard solution of potassium permanganate is run in, till the liquid acquires a pink tint surviving agitation and lasting some seconds   Further decolorisation may occur on standing, but this should be disregarded   Each centimetre of $\frac{1}{20}$ normal permanganate (= 1 578 gramme of $KMnO_4$ per litre) decolorised represents 0 00375 gramme of uric acid [1]   F G Hopkins strongly recommends this process (*Jour Pathology and Bacteriology*, June 1893) When it is intended to titrate the ammonium urate with standard permanganate in the above manner, it is very desirable to wash the precipitate with a saturated solution of ammonium sulphate, instead of ammonium chloride, since the latter salt somewhat affects the accuracy of the titration

The same method of titration by permanganate may be applied to the uric acid isolated in process 1, after simply dissolving it in a little hot solution of sodium carbonate

4 Bayrac (*Compt rend*, cx 352) determines uric acid by evaporating 50 c c of the urine to dryness at 100° C, treating the residue with 10 c c of dilute hydrochloric acid (1 5), and washing with alcohol to

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[1] This factor is due to F G Hopkins, as the result of experiment   As it corresponds to no simple reaction, the process has been investigated in the author's laboratory by A R Tankard   The first results on pure uric acid were not very constant, ranging from 96 to 104 per cent of the truth, but on taking as the end reaction the point at which the permanganate ceased to be *instantly* decolorised much closer figures were obtained   When the titration was conducted at a boiling heat, instead of at 60° C , the results were higher

remove urea and creatinine   The residue is dissolved
on the water-bath in 20 drops of caustic soda solution,
and heated to 90° or 100° C. with 15 c c of a concen-
trated solution of sodium hypobromite solution in
the apparatus for estimating urea shown in fig 11,
page 145   22 38 c c of nitrogen measured at 0° C and
760 mm   barometric pressure, or 23 55 c c at the
ordinary temperature and pressure, are said to repre-
sent 0 084 gramme of uric acid

Experiments made in the author's laboratory to
test the possibility of estimating uric acid by measur-
ing the amount of nitrogen evolved on treatment with
alkaline hypobromite have not yielded very encourag-
ing results, the reaction being subject to variations
from causes not yet understood

5   Uric acid can be determined by heating it with
concentrated sulphuric acid, and determining the am-
monia by treatment with hypobromite or distillation
with alkali   The method can be applied to a uric acid
precipitate   (See page 127 *et seq*)

F B Haycraft has described a method of deter-
mining uric acid based on its precipitation as silver
urate, on adding a solution of ammonio-nitrate of silver.
The process has been modified by Salkowski and
others   It is somewhat complex, and the results are
not more accurate than those yielded by methods
already detailed

F W Pavy (*Trans Royal Med and Chirurg
Soc , London*) has proposed to determine uric acid
volumetrically by an ammoniacal cupric solution, in
the manner so successfully employed for glucose (page
67), but the reducing power of uric acid on Pavy's
solution is found in practice to vary so greatly with
the method of conducting the titration and other
working conditions as to render the results of very
little value

Urates.

Uric acid is a feeble acid which is usually stated to possess a dibasic function. But it was shown by B e n c e J o n e s (*Jour Chem Soc*, xv 201), and has been confirmed more recently by S i r W m R o b e r t s (*Croonian Lectures*, 1892), that a third series of urates exist and have great physiological significance. The salts of the formula $M_2C_5H_2N_4O_3$ commonly called *neutral* or *normal urates*, dissolve readily in water, and are exclusively laboratory-products, not being met with in the animal system under either healthy or pathological conditions. The *acid urates*, or "bi-urates," of the formula $MHC_5H_2N_4O_3$ are very sparingly soluble, and exist in the urine only after it has undergone ammoniacal fermentation. They are known pathologically as components of gouty concretions in the tissues, but it is questionable if they ever exist physiologically in the blood or tissues. The third class, or "quadri-urates," have the composition $MHC_5H_2N_4O_3,H_2C_5H_2N_4O_3$. They are more soluble than the bi-urates, and are specially the physiological combinations of uric acid. They exist normally in the urine, and probably also in the blood, and constitute the whole of the urinary excretion of birds and serpents. Roberts considers that all the morbid phenomena due to uric acid probably arise from secondary changes in the quadri-urates.

Quadri-urates, $MHUr,H_2Ur$, usually present themselves as amorphous powders, but the spheres of birds' and serpents' urine are distinctly crystalline, and display a black cross when examined by polarised light. These forms are permanent in the air if kept perfectly dry, but readily assume a gelatinous character, and then appear under the microscope as large translucent globules. The quadri-urates are difficult to obtain pure. When produced artificially, they are apt to be mixed

with free uric acid or bi-urates, and when prepared from urine to be contaminated with pigments and traces of extraneous saline matters Roberts prepares potassium quadri-urate by adding 2 grammes of uric acid to a boiling solution of 9 grammes of potassium acetate in 300 c c of water The liquid is agitated for about a minute, filtered hot, and cooled rapidly in a stream of cold water The voluminous precipitate which forms is filtered off, washed in succession with rectified spirit and absolute alcohol, and dried at a temperature not exceeding 40° C The results of the analysis of the product obtained agree well with the formula $KHC_5H_2N_4O_3,H_2C_5H_2N_4O_3$ Other quadri-urates can be obtained by similar means, but they are less stable than the potassium salt

The quadri-urates are insoluble in alcohol, ether, chloroform, glycerin, and volatile oils, and cannot be dissolved without change in any simple menstruum. When treated with hot water, they pass momentarily into solution, but are almost immediately decomposed into bi-urate and free uric acid The same decomposition is effected by neutral saline solutions, but in this case, and notably with a solution of common salt, the decomposition is greatly retarded. When treated with solutions of alkaline carbonates, or disodium hydrogen phosphate, the quadri-urates are converted into bi-urates In healthy urine of feeble acid reaction the quadri-urates dissolve unchanged, but the solution undergoes gradual but complete decomposition, with ultimate separation of the whole of the uric acid in a free state This change is retarded in normal urine by the salts and colouring matters present (urea has no influence), occurring with greater facility the larger the proportion of free acid there is present

In studying the action of water on quadri-urates Sir W Roberts recommends that about 0 4 gramme of

the dried deposit should be stirred up with 1000 parts (= 400 c c) of distilled water, the mixture heated nearly to the boiling point until solution is complete, and then left at rest for forty-eight hours The supernatant liquid is then syphoned off and the remainder passed through a weighed filter The crystals of uric acid are washed very sparingly with cold water, then more freely with rectified spirit, dried, and weighed [1] A correction of 0 0055 gramme per 100 c c of mother liquor is applied to compensate for the solubility of the uric acid The decanted liquid, filtrate, and washings are next heated nearly to boiling, strongly acidulated with hydrochloric acid, and allowed to stand forty-eight hours as before

By the foregoing method, Sir W Roberts obtained the following figures from two specimens of quadri-urate prepared by the acetate of potassium method

	Sample A	Sample B
Uric acid separated by water (corrected for solubility),	0 080 gramme	0 164 gramme
Uric acid dissolved as bi-urate,	0 077 „	0 159 „

These results sufficiently establish the existence of the quadri-urate and the manner of its decomposition by water

The decomposition of sodium quadri-urate under the influence of water can be conveniently observed by filtering off the buff-coloured sediment deposited by healthy urine, washing it thoroughly with cold rectified spirit and drying it at a blood-heat When the quadri-urate thus purified is mixed with a considerable volume of water it is speedily disintegrated, a portion passing into solution in combination with the bases, and the remainder falling as an insoluble precipitate of crystalline uric acid The change is readily observed under the microscope by intimately mixing a particle

[1] It is evident that the modified methods of determining uric acid, described on page 168 et seq , may with advantage be employed here

of the purified deposit on a glass slide with a drop of water and protecting the mixture with a covering-glass In the course of ten minutes ovoid crystals of uric acid make their appearance, and grow and multiply till in the course of half an hour the entire field is thickly studded with crystals, the process continuing, provided that water be added as required, until the amorphous substance is entirely replaced by crystals of uric acid [1]

The quadri-urates readily assume a gelatinous form Thus, if a 5 per cent solution of ordinary sodium phosphate be heated to boiling with excess of uric acid, and the liquid filtered hot, the filtrate sets to a jelly on cooling This jelly, after being pressed between blotting-paper to free it from mother liquor, exhibits the characteristic behaviour of a quadri-urate, being rapidly decomposed by water with copious formation of crystals of uric acid On keeping in a moist condition, gelatinous sodium quadri-urate gradually passes into a crystalline condition, and then appears under the microscope in radiating spheres, exactly similar to the spheres so common in serpents' and birds' urine

If the white mortar-like substance which constitutes the urinary excretion of birds and serpents be examined in its fresh and uncontaminated state, and not after contact with water or bacterial fermentation, it will be found to behave in an exactly similar manner to artificial quadri-urates Under the microscope it appears as minute spheres, which exhibit a radiated structure and display a black cross with polarised light On adding a drop of water, the spheres are seen gradually to melt away, with formation of hexagonal tablets of uric acid

BI-URATES have the general formula $MHC_5H_2N_4O_3$,

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[1] Crystals of the sodium bi urate simultaneously formed are never observed, since this salt is liberated in the gelatinous form

or MHUr   They result from the action of water on
the quadri-urates, and exist in the body under various
pathological conditions   The sodium salt, which is the
most important and characteristic member of the series,
possesses the following properties —

Acid Sodium Urate, or *Sodium Bi-urate*, contains
$2(NaHC_5H_2N_4O_3) + H_2O$. It generally forms a crystalline
powder, which, under the microscope,
appears in needles (often crossed),
rosettes, stellate, and hedgehog-like
forms   It requires about 1200 parts
of cold or 120 of boiling water for
solution   Sodium bi-urate is readily
obtained by passing carbon di-oxide
through a solution of uric acid in
caustic soda, or by boiling uric acid
with sodium carbonate, phosphate, or
acetate, or with borax   The buff or brick-red sediment

Fig   17 — ACID
SODIUM   URATE
(After Frey)

often thrown down by urine is commonly stated to
consist of sodium bi-urate, but R o b e r t s has shown
that it consists essentially of sodium quadri-urate
(page 175)

The solubility of acid urate of sodium in water im-\
pregnated with salt and other substances has an im-
portant bearing on the cause and cure of gout, and has
been investigated by Sir Wm Roberts, who gives the
figures on next page, which represent the parts by
weight of sodium urate dissolved at 100° F (= 37 8° C)
by 1000 parts of the solution of the strengths indicated
The amount of sodium bi-urate dissolved by 1000 parts
of distilled water at 100° F was found to be 1 0   From
the following results it appears that the solvent action
of the various salts depends on the nature of the metal,
and has no reference to its form of combination   Salts
having an alkaline reaction to litmus, like the car-
bonates and phosphates, behave exactly similarly to

M

those of neutral reaction, such as the chlorides and sulphates   Salts of potassium exert no appreciable influence on the solubility of sodium bi-urate in water   Salts of sodium decrease the solubility, the influence being greater the larger the proportion of salt present. Salts of ammonium, calcium, and magnesium behave similarly to, but less powerfully than, salts of sodium

Percentage of Salt in Solvent	0 1 per cent	0 2 per cent.	0 3 per cent	0 5 per cent.	0 7 per cent	1 0 per cent
Sodium bicarbonate,	0 50	0 34	0 20	0 13	0 09	0 08
Sodium chloride,	0 45	0 30	0 16	0 10	0 08	0 05
Sodium phosphate (crystallised),	0 70			0 32		
Sodium sulphate (crystallised),	0 55			0 24		
Sodium salicylate,	0 65		0 36	0 25		
Potassium bicarbonate,	0 96	1 00	1 00	0 97	1 02	0 98
Potassium chloride,	0 96		1 01	1 10		
Potassium phosphate,	1 01			1 00		
Ammonium chloride,	0 85	0 50	0 42	0 35		
Calcium chloride,				0 27		
Calcium sulphate,	0 65	0 44				
Magnesium chloride,	0 85			0 68		
Magnesium sulphate (crystallised),	0 90					

Crystalline sodium bi-urate is ten times as soluble in boiling water as in cold, but a saturated hot solution does not deposit the excess of salt immediately on cooling   The bi-urate remains in complete solution for a considerable time, and is not entirely deposited for some days   Roberts has shown that this behaviour is not merely due to supersaturation of the liquid, but is owing to the formation of a gelatinous modification of the bi-urate of greater solubility than the crystalline form   Thus if a saturated solution of sodium bi-urate in boiling water be prepared, and when cold mixed with an equal measure of a 20 per cent solution of common salt, a voluminous gelatinous precipitate will be thrown down   Saturated solutions or solid crystals of other salts (e g , sodium phosphate or acetate, potassium chloride, phosphate, acetate, &c) may be substituted for the common salt   The precipitate, if filtered off,

allowed to drain, and cautiously washed with cold water, consists of sodium bi-urate in a state of approximate purity   It dissolves at 100° F in blood-serum, or in a liquid containing 0 5 gramme of sodium chloride and 0 2 of sodium carbonate per 100 c c (which represents the saline ingredients of serum), sufficiently freely to cause a considerable separation of uric acid after acidulating with acetic acid , whereas crystalline sodium bi-urate is taken up by water at 100° F so slightly that no deposition of uric acid is obtainable on acidulating the liquid

The gelatinous form of sodium bi-urate gradually changes into the crystalline variety, and the gradual deposition of the salt from its solution in water blood-serum, or imitation-serum is evidently due to the same change of condition

Acid Potassium Urate, or *Potassium Bi-urate*, KHUr, is said to be sometimes formed as a urinary deposit in cases of fever   It is amorphous, and more soluble than the corresponding sodium salt, requiring for solution only 800 parts of cold or 70 to 80 parts of boiling water

Acid Lithium Urate or *Lithium Bi-urate*, LiHUr, forms crystalline grains, soluble in 370 parts of cold or 39 of boiling water   L i p o w i t z states that if equal parts of uric acid and lithium carbonate be treated with 90 parts of water at blood-heat, a clear solution is obtained, while at 100° C four times the amount of uric acid can be dissolved without increasing the weight of lithium carbonate   Seeing that lithium carbonate itself requires about 200 parts of water for solution, its solvent action on uric acid is remarkable, and is of much interest in connection with the extensive application of lithium salts in the treatment of gout   On the other hand, it is stated by L S i e b o l d (*Year-book Pharm*, 1889, page 413) as

the result of direct experiment, that the relative
solvent action of solutions of lithium, sodium, and
potassium carbonates on a given weight of uric acid,
under equal conditions of dilution and at a temperature
of 37° C (blood-heat), is strictly proportional to the
ratio of the molecular weight of these solvents   Hence
lithium carbonate has the advantage that 74 parts
are chemically equivalent to 106 of the sodium salt
or 138 of potassium carbonate , but there the advan-
tage ceases   Urinary sediments are similarly dissolved
by these carbonates with equal facility if molecular
proportions are used, and equivalent weights of the
citrates of lithium, sodium, and potassium produce
equal alkalinity in the urine of the person taking
them   Siebold further states that lithium chloride
and sulphate have no solvent action on uric acid and
acid urates, and that natural mineral waters contain-
ing these salts have none beyond that exercised by
basic constituents simultaneously present, and by the
water

Acid Ammonium Urate, $(NH_4)HU_1$, is soluble in
about 1500 parts of cold water, and quite insoluble
in saturated solutions of ammonium chloride and sul-
phate (compare page 168)   The urinary excrement
of serpents is commonly stated to consist almost
wholly of a mixture of acid urate of ammonium
with free uric acid   This is often true of the altered
product, but Sir W Roberts has shown that, in a fresh
and undecomposed state, serpents' urine consists sub-
stantially of quadri-urates, which undergo decompo-
sition into a mixture of bi-urates and free uric acid by
contact with water (page 176)   Guano, the excrement
of various aquatic birds, consists chiefly of oxalate and
acid urate of ammonium in admixture with phosphates
G u a n i n e, $C_5H_5N_5O$, a base forming crystallisable
salts with acids, is also a constituent of guano, and

replaces uric acid in the urine of spiders and other invertebrate animals

NEUTRAL or NORMAL URATES of the light metals do not exist naturally, but they may be obtained by dissolving uric acid in the theoretical amount of alkali The normal urates of lithium and ammonium are unknown *Neutral Potassium Urate*, $K_2C_5H_2N_4O_3$, forms small crystals having an alkaline reaction and caustic taste It dissolves, with partial decomposition into the acid salt, in about 36 parts of cold water, forming a liquid of soapy taste which froths strongly when shaken *Normal Sodium Urate*, $Na_2U_1 + H_2O$, forms hard nodules which closely resemble the potassium salt, but are less soluble in water

On passing carbon dioxide through a solution of the normal urate of potassium or sodium the corresponding a c i d u r a t e is precipitated The same decomposition occurs by prolonged boiling of the solution

The following table, due to R a l p h (*Practical Treatise on Diseases of the Kidneys*, 1885), shows the characters of the urates of the light metals —

Urate	Solubility in Cold Water	Character of Deposit
Potassium, acid,	1–800	Amorphous
,, normal,	1–44	Amorphous, or in fine needles
Sodium , acid,	1–1200	Amorphous , rarely crystalline
,, normal,	1–77	Nodular masses
Lithium , acid,	1–60	Amorphous, or in fine needles
Calcium , acid, .	1–600	Amorphous, or in fine needles
,, normal,	1–1500	Fine granules
Ammonium ; acid,	1–1600	Amorphous, or spiked globular masses

The urates of lead, copper, mercury, and silver are quite insoluble in water Hence solutions of these metals are used for determining uric acid or for separating urates from urine

The behaviour of the urates with water and saline solutions has an important bearing on the cause and treatment of gout   It is probable that in media containing alkaline carbonates—such as the serum of the blood, and its derivatives lymph and synovia—uric acid passes into solution in the first instance as quadri-urate, and it may be inferred that it circulates in the blood and is voided in the urine in the same form   In perfect health, the elimination of the quadri-urate proceeds with sufficient speed and completeness to prevent any undue detention or any accumulation of it in the blood   But in gouty subjects, either from defective action of the kidneys or from excessive introduction of uric acid into the circulation, the quadri-urate lingers unduly in the blood and accumulates therein   The detained quadri-urate, circulating in a medium rich in carbonate of sodium, gradually takes up an additional atom of base, and is thereby converted into bi-urate, which at first exists in the hydrated or gelatinous condition, but with lapse of time and accumulation passes into the insoluble crystalline condition, and then symptoms of gout manifest themselves

A Haig (*Med Chirurg Trans*, lxxi 125, 283) has shown that administration of acids diminishes the relative amount of uric acid excreted, while that of alkalies increases it   Thus the normal proportion of uric acid to urea is 1 35, but after a few doses of citric acid the relation was 1 41, and after similar doses of potassium citrate 1 28   In these cases there was not only a relative but also an absolute diminution and increase in the uric acid excreted   Salicylic acid forms an important exception to the general behaviour of acids, for while it increases urinary activity it does not in any way diminish the excretion of uric acid   Moreover, acids given while

salicylates are present in the circulation have no longer the power of diminishing the excretion of uric acid, nor is excessive excretion of uric acid under salicylates accompanied by any headache   Both uric and salicyluric acids are present in the urine passed under the influence of salicylates, probably owing to the salicylate acting on the uric acid in the blood, but not on that secreted by the kidney itself   Benzoates do not act in the same way as salicylates, probably because the hippuric acid formed from them is less soluble than salicyluric acid   The value of salicylates in uric acid diseases is largely due to their power of preventing acids from causing retention of uric acid   Thus salicylates prevent gout, the peculiar headache due to uric acid and frequent after breakfast, and also epilepsy, which is probably due to uric acid acting on the nerve-centres

Hippuric Acid. Benzoyl-amidoacetic Acid   Benzoyl-glycocine $C_9H_9NO_3$, or, C_6H_5 CO NH CH_2 COOH

Hippuric acid derives its interest less from its pathological importance than as affording a typical example of the so-called "conjugated bodies" of which the synthesis is readily effected within the living organism   Thus if benzoic acid be taken internally, it appears in the urine as hippuric acid, and hippuric acid may be obtained artificially by heating benzoic anhydride with amido-acetic acid (glycocine), or the zinc salt of the latter with benzoyl chloride —

$$C_7H_5O\ Cl + C_2H_2(NH_2)O\ OH = C_7H_5O\ O\ C_2H_2(NH_2)O + HCl$$

Benzoyl Chloride   Glycocine   Hippuric Acid
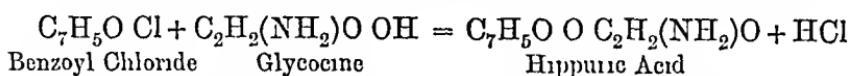
Benzoic aldehyde, toluene, cinnamic acid, quinic acid and phenyl-propionic acid when ingested, are also excreted as hippuric acid   Substituted benzoic acids appear in the urine as substituted hippuric acids   Salicylic acid, which has the constitution of

ortho-hydroxybenzoic acid, $C_6H_4(OH)COOH$, is converted in the system into h y d r o x y - h i p p u r i c or s a l i c y l u r i c   a c i d, $C_9H_8(OH)NO_3$, which may be detected in the urine by the bluish-violet coloration produced on adding dilute ferric chloride

The quantity of hippuric acid excreted in normal human urine is stated to range from 5 to 60 grains ($= 0.3$ to 3.8 grammes) in twenty-four hours, but an increase results from a vegetable diet   This has been particularly noticed after eating plums, pears and cranberries, and the cuticular parts of many plants act similarly   In the urine of diabetic patients, hippuric acid is frequently present in much increased proportion, as also in jaundice and other liver-complaints, and it is abundant in the acid urine of persons suffering from all kinds of fevers

Hippuric acid replaces uric acid in the urine of herbivorous animals, which are stated to contain it to the extent of about 2 per cent ,[1] its origin being doubtless in bodies of the aromatic series existent in the food Hippuric acid is also found in the excrement of the lower animals, except that of birds, which contains the allied substance o r n i t h u r i c   a c i d, having the constitution of a d i b e n z o y l - d i a m i d o v a l e r i c 'a c i d :[2]—

$$C_6H_5 CO NH \atop C_6H_5 CO NH \Big\} \ C_4H_7 COOH$$
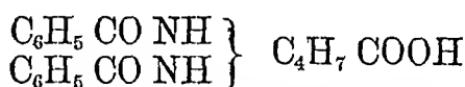
[1] Hippuric acid may be conveniently prepared from *fresh* cows' or horses' urine, which often contains sufficient to yield a precipitate on mere addition of excess of hydrochloric acid   If not, the urine should be boiled with milk of lime, and the filtrate neutralised, concentrated, and treated with excess of hydrochloric acid , or the neutralised filtrate may be precipitated with ferric chloride, and the washed precipitate decomposed by hydrochloric acid   The hippuric acid is freed from colouring matter by recrystallising it from chlorine water, or by treating it with bleaching powder and hydrochloric acid

[2] On boiling ornithuric acid with hydrochloric acid, it almost immediately parts with one benzoyl group and yields b e n z o y l - o r n i t h i n e, which on further boiling splits up into benzoic acid and diamido valeric acid or o r n i t h i n e, $(NH_2)_2C_4H_7 COOH$, a base of strong alkaline reaction and of caustic taste

Hippuric acid crystallises in milk-white rhombic prisms ending in two or four facets, the crystals being often grouped in clumps (fig 18)   As liberated by adding hydrochloric acid to cows' urine or one of its salts, hippuric acid is apt to form ill-defined crystals

Hippuric acid has a slightly bitter taste, free from acidity   It melts at 187 5° C, and above 240° decomposes, with an odour of hay or fresh urine, and formation of h y d r o - c y a n i c and b e n z o i c a c i d s and b e n z o n i t r i l

Fig 18 — HIPPURIC ACID (after Frey) a, a, Prisms , b, Crystals formed by slow evaporation

(phenyl cyanide), $C_6H_5 CN$, a dark resinous or coaly mass being left

Hippuric acid requires about 600 parts of ice-cold water for solution, but dissolves tolerably readily in hot water   It is also soluble in alcohol, especially when hot The aqueous and alcoholic solutions have an acid reaction   Hippuric acid is but slightly soluble in cold ether, but dissolves in acetic ether, and readily in boiling amylic alcohol   In chloroform, benzene, petroleum spirit, and carbon disulphide it is practically insoluble

When boiled for a time (half an hour) with dilute nitric, hydrochloric, or oxalic acid (or more rapidly if strong hydrochloric acid be used), hippuric acid undergoes hydrolysis, the liquid on cooling depositing b e n z o i c a c i d, while a salt of g l y c o c i n e (amido-acetic acid) remains in solution — $C_9H_3NO_3 + H_2O = C_7H_6O_2 + C_2H_5NO_2$ A similar reaction takes place spontaneously in urine containing hippuric acid, under the influence of ferments   Hence only perfectly fresh urine will yield hippuric acid   If the urine be

alkaline, as is usually the case with that of herbivorous mammals, the glycocine first produced splits up into ammonia and acetic acid —$C_2H_5NO_2 + H_2O = C_2H_4O_2 + NH_3$

If hippuric acid be evaporated to dryness with concentrated nitric acid, and the residue heated, an odour of nitrobenzene is evolved

Hippuric acid decomposes carbonates, and dissolves zinc with evolution of hydrogen   Its salts are mostly soluble and crystallisable   The hippurates of silver, lead, and copper are sparingly soluble.

When ferric chloride is added to a solution of a hippurate, a cream-coloured precipitate of f e r r i c h i p p u r a t e is thrown down, which contains more or less basic salt, according to the greater or less dilution of the solution   The precipitate is almost insoluble in pure water, but dissolves in free hippuric acid, in excess of ferric chloride, and in alcohol   The reaction with ferric salts may be employed for the determination of hippuric acid in urine   For this purpose, the urine is acidulated with nitric acid, heated to boiling to remove carbon dioxide, neutralised with calcium carbonate, treated with excess of lead nitrate, and then diluted to a known volume and filtered   An aliquot part of the filtrate is then heated and titrated with a solution of neutral ferric nitrate which has been standardised with pure hippuric acid   The reaction is at an end when a drop of the clear liquid gives a blue coloration with potassium ferrocyanide The distinction between this point and the previous formation of the white ferrocyanide of lead is very sharp

On treating ferric hippurate or the solution of a soluble hippurate with excess of hydrochloric acid, the hippuric acid separates sooner or later in long crystalline needles   Hippuric acid is distinguished from

benzoic and salicylic acids by its crystalline form (figs 18, 19, pages 185, 187), by charring when heated with strong sulphuric acid, by giving off ammonia on ignition with soda-lime, and by not being dissolved on agitating its solution with chloroform or petroleum-spirit

For the detection of hippuric acid in the urine of herbivora, the fresh liquid should be treated with milk of lime, filtered, the filtrate concentrated to a syrup as rapidly as possible, and excess of hydrochloric acid added, when hippuric acid crystallises out on standing   If the urine be at all stale, benzoic acid will be obtained instead, and may be distinguished from hippuric acid as just described

Fig 19 —BENZOIC ACID

For the detection of traces of hippuric acid in human urine, the perfectly fresh liquid should be evaporated nearly to dryness at 100° C, the residue mixed with powdered barium sulphate, a little hydrochloric acid added, and the whole exhausted with rectified spirit   The alcoholic solution is carefully neutralised with soda, and the liquid evaporated   The residue is mixed with a little oxalic acid, and again evaporated to dryness on the water-bath The residue is exhausted with a mixture of equal measures of alcohol and ether, the solution distilled to a small bulk, boiled with milk of lime, filtered, concentrated, and acidulated with hydrochloric acid Immediately, or on standing, according to the quantity present, crystalline needles of hippuric acid separate, and may be filtered off and purified by washing, first with diluted hydrochloric acid and then with a little ether

Another method is to precipitate the urine (1000 to
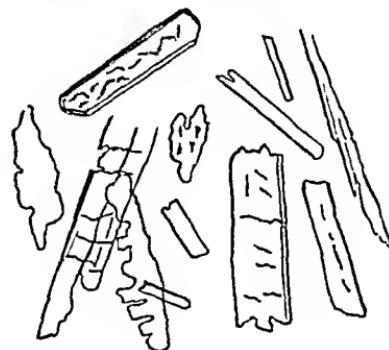
1200 c c) with a slight excess of strong baryta-water The filtered liquid is treated with dilute sulphuric acid till exactly neutral to litmus, decanted or filtered from the precipitated barium sulphate, and evaporated to a syrup on the water-bath The residue, which should be exactly neutral, is treated while still hot with 150 to 200 c c of absolute alcohol and thoroughly agitated Barium succinate, sodium chloride, and other compounds are thus precipitated The liquid is decanted, the alcohol evaporated, and the syrupy residue treated while still hot with hydrochloric acid The liberated hippuric acid is extracted by repeated agitations with ether (100 to 150 c c), the separated ether distilled off, the residue diluted with water, and heated to boiling with a little milk of lime The liquid is filtered, concentrated, and treated with excess of hydrochloric acid, when hippuric acid separates in fine crystals, which can be obtained colourless by treatment with purified animal charcoal

COLOURING MATTERS OF URINE.

THE colour of urine is liable to modification under the influence of a variety of pathological conditions   The following *résumé* is based on that of Krukenberg —

Nearly colourless or pale yellow urine may be due either to greater dilution or to diminution of the normal pigments   It occurs specially in anæmia chlorosis, diabetes, granular kidney, &c

Yellowish and milky urine may be due to the presence of floating globules of fat, as in chyluria , or to suspended pus-corpuscles, as in pyelitis or other purulent disease of the urinary tract

Orange-coloured urine is excreted after the ingestion of certain drugs, such as chrysophanic acid, rhubarb, santonin, &c [1]

Brownish-yellow to red-brown urine, which becomes blood-red on addition of an alkali, is likewise indicative of excreted drugs, such as rhubarb, senna, chelidonium, &c   Port-wine coloured urine is sometimes excreted after sulphonal has been taken

Dark yellow to brown-red urine, which easily deposits a sediment, occurs in certain febrile diseases, and owes its colour to an increase of the normal pigments or to the occurrence of pathological colouring matters

Red or reddish urine may be due to pigmentary

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[1] If the urine be treated with caustic alkali and then shaken with amylic alcohol, the colouring matter of santonin is dissolved out, and becomes yellow on exposure to air   The colouring matter of rhubarb (chrysophanic acid) is not sensibly dissolved by amylic alcohol

matters (e g , logwood, madder, bilberries, coal-tar dyes) in the food, or to the presence of unchanged hæmoglobin, occurring in hæmoglobinuria or in urinary hæmorrhage   A *brown* colour may also be due to hæmorrhage into the kidneys or to methæmoglobinuria

Brown or brownish-black urine is excreted in cases of methæmoglobinuria and hæmorrhage, and is especially indicative of melanitic sarcoma.   It is also highly characteristic of poisoning by carbolic acid, in which case hydrochinone and catechol (pyrocatechin) appear in the urine.[1]

Yellowish-green, green, or greenish-brown urine is indicative of the presence of bile-pigments, and of course it occurs in jaundice and other affections of the liver

Dirty green or blue urine, sometimes showing a dark *blue scum* with a *blue deposit*, indicates the presence of excess of indigogens.   It frequently occurs in cholera and typhus,[2] especially when the urine is putrefying

The chemistry of urochromes is still in a very confused state, notwithstanding the great attention the subject has received   An exhaustive recapitulation of

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[1] Melanitic urine, on addition of ferric chloride, yields a brown turbidity or black precipitate soluble in excess   On adding a dilute solution of sodium nitroprusside, followed by caustic alkali, they frequently give a pink or red coloration, and on adding an acid Prussian blue is precipitated

[2] The urine of healthy persons gives a negative reaction with the "diazo test," while, on the other hand, the urine of patients suffering from typhus and certain other fevers exhibits the reaction in a marked manner   The reaction is also produced in cases of acute tuberculosis, but not by the urine in intestinal catarrh, which can thus be differentiated from typhus fever   (See Rutimeyer, *Lancet*, ii , 1890, page 413)   To apply the diazo test, 1 gramme of sulphanilic acid is dissolved in 10 c c of pure hydrochloric acid, and the solution diluted with water to 200 c c   Fifty c c of this reagent is mixed with 5 c c of a solution of 1 gramme of potassium or sodium nitrite ($NaNO_2$) in 200 c c of water, and 50 c c of the sample of urine added   The mixture is made strongly alkaline by ammonia, and the whole well shaken   A bright red colour is produced by the pathological urines above mentioned, and on standing for twenty-four hours a deposit forms, the upper part of which is green or black   Healthy urine gives no such reaction

the existing knowledge on the subject is beyond the scope of this work, but the following is a brief outline of the leading facts known respecting the principal colouring matters of normal and pathological urine

Urobilin.

The best known and most definite of the colouring matters of normal urine is *urobilin*, to which the formula $C_{32}H_{40}N_4O_7$ or $2C_{16}H_{18}N_2O_3 + H_2O$ is ascribed It is a yellowish-brown amorphous substance, almost insoluble in pure water but soluble in presence of small quantities of free acids or neutral salts, slightly soluble in ether and benzene, and readily so in alcohol and chloroform The neutral alcoholic solution is reddish-yellow, and when concentrated exhibits a green fluorescence On addition of acid, the fluorescence is destroyed, and the dilute solution acquires a rose tint Alkaline solutions of urobilin are yellowish or yellowish-green, according to their concentration, and show a fluorescence which is much more marked after addition of a solution of zinc chloride, the liquid then appearing rose-coloured by transmitted and green by reflected light Neutral and alkaline solutions of urobilin in alcohol, when examined in the spectroscope, exhibit an absorption-band between the Fraunhofer-lines b and F The absorption is much increased by the addition of zinc chloride Normal urobilin is regarded by MacMunn as an oxidation-product of effete hæmatin and bile-pigments and not as a reduction-product It appears to be identical with *choletelin*, a body resulting from the treatment of hæmatin in acid solution with hydrogen peroxide, and said by M a l y to have the formula $C_{16}H_{18}N_2O_6$

The proportion of urobilin in fresh normal urine is often extremely small, but appears to increase on

exposure of the urine to the air This behaviour is probably due to the presence in the urine of a chromogen or mother-substance, which is converted into urobilin by oxidation

For the extraction and estimation of urobilin, A Studensky (*Chem Centr*, 1893, ii 668) recommends that 20 c c of the urine should be treated with 2 c c of a saturated solution of cupric sulphate, the liquid saturated with crystallised ammonium sulphate, 10 c c of chloroform added, and the mixture shaken for some minutes The urobilin dissolves in the chloroform with copper-red colour, while bilirubin and its allies are not extracted in presence of copper sulphate A portion of the chloroformic layer is tapped off, and its colour compared with that of a standard solution of urobilin, prepared in a similar manner from a large quantity of urine The chloroformic solution thus obtained is evaporated to dryness, the residue washed with ether, and weighed Standard solutions of urobilin are prepared by dissolving known weights of this residue in chloroform, and the solution yielded by the sample under examination is compared with them The standard solutions remain unchanged for some months, if preserved in the dark in closed vessels, and covered with a layer of saturated solution of ammonium sulphate

PATHOLOGICAL UROBILIN is, according to MacMunn, a substance distinct from normal urobilin, though obtainable in the same manner and soluble in the same menstrua as that body The absorption-band at F, which characterises the spectrum of the urobilin series, is much broader and darker in the case of the pathological than in that of the normal colouring matter, and other characteristic differences are recognised by MacMunn, who regards the former as less oxidised than the normal product and indistinguish-

able from and probably identical with s t e r c o b i l i n, the colouring matter of fæces [1]

UROERYTHRIN is the colouring matter of certain bright pink urinary deposits, and possibly of the highly coloured urine excreted in rheumatism It may be extracted by boiling pink urates with alcohol The solution exhibits two ill-defined absorption-bands between D and F, and the residue left on evaporation is turned green by caustic alkalies

UROMELANIN is a dark-coloured pigment produced by boiling urine with hydrochloric acid According to L von Udiansky, it has its origin in the carbohydrates of the urine (*Zeitsch physiol Chem*, xi 537 , xii 33)

URO-EOSEIN is the name given to an extremely unstable pigment observed by N e n c k i and S i e b e r in diabetic urine which became bright pink on adding hydrochloric acid It was extracted by amylic alcohol, and showed a characteristic absorption-band between D and E

Bile Pigments in Urine.

The colouring matters characteristic of bile are not present in normal urine, but in certain diseases (jaundice, &c) they exist in very appreciable amount Such urine exhibits a yellowish-green, green, greenish-brown, or almost black colour The most characteristic colouring matter of bile is *bilirubin*, $C_{16}H_{18}N_2O_3$, while *biliverdin, biliprasin, bilifuscin, bilicyanin,* &c , are

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[1] MacMunn points out that, "if this be so, then the presence of pathological urobilin in urine is, to a certain extent, an indication of the absorption of fecal matters from the intestine, and with them of poisonous alkaloidal bodies—ptomaines, which have escaped the destructive action of the liver By such an assumption we can explain many of the symptoms which accompany its presence in urine   It would appear that, in those cases where pathological urobilin occurs in urine, there is either some vaso motor disturbance of the hepatic circulation, or some mechanical interference with it, as in passive congestion '

products of its oxidation   In bilious urine of saffron-yellow colour bilirubin predominates, while biliverdin and other oxidation-products are present in greenish urine   Bilious urine gives a yellow froth on agitation, and stains linen and filter-paper yellow [1]   Bilirubin may be extracted from its acidulated solutions by agitation with chloroform   It dissolves in caustic alkalies, and on exposing the solution to the air it is converted into b i l i v e r d i n, $C_{16}H_{18}N_2O_4$, subsequently into b i l i p r a s i n, $C_{16}H_{22}N_2O_6$, and, according to M a l y, ultimately into c h o l e t i l i n, $C_{16}H_{18}N_2O_6$

A variety of tests have been proposed for the detection of bile-pigments in urine, but the following are the most delicate and answer every purpose —

Gmelin's Test consists in treating the urine with strong nitric acid and observing the change of colour produced   The reaction is best observed by allowing some of the urine to run gently onto the surface of some fuming nitric acid contained in a test-tube   If bile-pigments be present, a green ring will become apparent at the point of contact, while below this will appear violet, red, and yellow zones, in the order named   The green colour is alone characteristic of bilious urine, since indigogens give rise to blue and red colorations

Various modifications of Gmelin's reaction have been proposed, but they possess no advantage over the above mode of applying the test

Rosin's Test for bile-pigments consists in allowing very dilute iodine solution or bromine-water to flow on to the surface of the urine from a pipette   A grass-green ring is produced at the junction of the two strata

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[1] If the dyed filter-paper be treated with a drop of nitric acid, the margin of the spot will become violet or deep blue, while the centre gradually changes to emerald green

For the detection of *traces* of bile-pigments, often of great clinical and physiological importance,[1] the urine should be treated with a moderate excess of lime-water, and the excess of lime precipitated as carbonate, by passing carbon dioxide gas or adding seltzer-water, till the liquid no longer exhibits an alkaline reaction to litmus (or preferably to phenolphthalein) The precipitate is collected on a filter, and treated with fuming nitric acid, when the green and other colours already described will become evident if bile be present. Or the precipitate may be boiled with alcohol acidulated with sulphuric acid, when the supernatant liquid will acquire a grass-green colour, a white deposit of calcium sulphate being simultaneously formed

Another reliable test for bile-pigments in urine is to treat 30 c c (or 1 oz) of the sample with about one-third of its bulk of a 20 per cent solution of zinc acetate,[2] after previously neutralising most of the free acid by sodium carbonate. The voluminous precipitate is filtered off, washed, and treated with a little ammonia. In presence of bile-pigments the ammoniacal liquid is usually fluorescent, and either at once or on standing shows the absorption-spectrum of b i l i c y a n i n, characterised by bands on each side of the D line, and a third between *b* and F

[1] In two cases of highly icteric urine, after the occurrence of ammoniacal fermentation Salkowski could detect no bilirubin by Gmelin's test, and extraction yielded no unchanged biliary pigment He suggests that this decomposition of bilirubin without the formation of any characteristic products was probably the result of the activity of bacteria, and may explain other cases of jaundice, in which the urine, though dark coloured, gave no evidence of the presence of bile pigments

[2] Zinc acetate may be readily extemporised by treating lead acetate with zinc sulphate in slight excess, and filtering from the precipitated lead sulphate Or sodium acetate may be added to a solution of zinc sulphate or chloride, the sodium sulphate or chloride formed simultaneously with the zinc acetate being disregarded

Blood Pigments in Urine.

Blood occurs in urine in certain diseases In *Hæmaturia* blood is present, and blood-corpuscles may be recognised by examining the deposit formed on standing under a high microscopic power. In *Hæmoglobinuria*, the urine contains the red colouring matter of the blood, but no actual blood-corpuscles unless blood also be present

The micro-spectroscope affords the most satisfactory means of detecting blood-pigments in urine In its absence, the following tests may be applied —

Heller's Test consists in making the urine strongly alkaline with caustic soda or potash and heating the liquid to boiling In presence of blood a bottle-green coloration is produced, and a brownish-red precipitate, consisting of earthy phosphates coloured by blood, is thrown down

Hæmin Test —The deposit which separates from the urine on standing is evaporated to dryness at a gentle heat with a small fragment of common salt The residue is treated with two or three drops of glacial acetic acid, and heated on a glass microscope-slide On cooling, if blood-pigments were present, microscopic crystals of hæmin as reddish-brown rhomboidal plates will be observed

According to C Rosenthal (*Chem. Centr.*, 1886, page 251), Heller's test for hæmoglobin, based on the red coloration produced by warming with aqueous soda, fails when the proportion of blood is less than 1 part per 1000 of the urine Struve's test, consisting in the isolation of hæmin from the precipitate produced by tannin, is uncertain in its results, but the presence of more than a *minute* trace of iron in the ash resulting from the ignition of this precipitate affords satisfactory evidence of the presence of hæmoglobin in the urine

UROHÆMATOPORPHORIN is regarded by M a c M u n n as solely a reduction-product of hæmatin, which has been produced in the organism by reduction of effete hæmoglobin or effete histo-hæmatin. It can be prepared artificially by the action of sodium-amalgam, zinc and dilute acid, and other reducing agents on hæmatin. In acid solutions, the spectrum exhibits a narrow absorption-band almost coincident with the D line, and another darker band between D and E, besides a feeble shading between these two, and a band at F closely resembling that of urobilin. If the alcoholic solution of the isolated colouring matter be treated with ammonia, the liquid shows a five-banded spectrum closely resembling that of h æ m a t o p o r p h o r i n.

HÆMATOPORPHYRIN is a colouring matter produced by the action of strong sulphuric acid on hæmatin or hæmin. According to Nencki and Siebel's first accounts it contains $C_{32}H_{34}N_4O_5$, but in a more recent research (*Monatsh*, ix 115) they regard this body as either a mixture or an anhydride of true h æ m a t o-p o r p h y r i n, which they prepare by acting on hæmin with a saturated solution of hydrobromic acid gas in glacial acetic acid. To this body they ascribe the formula $C_{16}H_{18}N_2O_3$, identical with that of anhydrous b i l i r u b i n, which body hæmatoporphyrin resembles in many of its properties. When introduced into the system, hæmatoporphyrin is partly expelled in the urine, but the greater portion is retained and probably utilised in the formation of hæmoglobin.

Hæmatoporphyrin is very frequently present in the dark-coloured urine excreted after the administration of sulphonal. O Hammersten (*Jour Chem Soc*, lxii 649 and 1136) examined four samples of urine from patients to whom sulphonal had been administered, and found hæmatoporphyrin in each case, but adds that more observations will be

needed before it can be positively stated that sulphonal is the cause of the appearance of hæmatoporphyrin in the excretion [1] For the detection of hæmatoporphyrin, Hammersten precipitates the urine with barium acetate and filters The filtrate is precipitated alternately with barium acetate and sodium carbonate until a small filtered portion gives a white precipitate with these reagents The hæmatoporphyrin is carried down in the precipitate Both precipitates are washed well and extracted with acidified alcohol The acid alcoholic solution is diluted with several times its measure of water, and shaken with chloroform, which extracts most of the colouring matter The chloroformic layer is rapidly tapped off from the upper stratum, washed well with water, and evaporated in shallow basins in the dark According to Hammersten, the brown residue left after evaporation is soluble with splendid purple colour in chloroform, insoluble in cold water and in very dilute acids, sparingly soluble in cold alcohol, but soluble in hot alcohol, from which it crystallises in needles resembling those of the hæmatoporphyrin hydrochloride of Nencki and Sieber On spectroscopic examination, the absorption-bands were found to be slightly nearer to the red end of the spectrum than those of the hæmatoporphyrin obtained by Nencki and Sieber In only one case out of the four did the substance appear to be absolutely identical with their product In another case the chromogen of a similar colouring matter was met with A solution of hæmatoporphyrin in ammonia and zinc chloride gives four absorption-bands. The two lying between C and D and between b and F disappear within twenty four hours, the former first The other two bands are permanent

[1] Hammersten's suggestion has been fully confirmed by Salkowski and others

Urinary Indigogens.

Normal urine contains traces of the potassium salt of indoxyl-sulphuric acid, $C_8H_6N SO_4H$ [1] This body is derived primarily from indole, C_8H_7N, which by oxidation yields indoxyl, $C_8H_5(NH) OH$ By reaction with the elements of sulphuric acid this is converted into an ethereal salt, the potassium compound of which is the substance in question

POTASSIUM INDOXYL-SULPHATE, $C_8H_6N SO_4K$, has received the unfortunate name of "urinary indican," from a supposed identity with plant-indican, the glucoside from which indigo is obtained The only similarity between the two bodies is that both yield indigo-blue as one of the products of their decomposition [2]

Potassium indoxyl-sulphate crystallises from hot alcohol in colourless lustrous tables, readily soluble in water but only sparingly in cold alcohol When boiled with dilute acid it is decomposed into indoxyl and acid potassium sulphate, but is not attacked by alkalies When the crystals are heated, indigotin (indigo-blue) sublimes, and the same substance is found quantitatively when the acidulated solution is warmed with ferric chloride

For the detection of indoxyl-sulphuric acid in urine, Jaffe (*Pfluger's Archiv*, iii 448) first separates any albumin by boiling the liquid, and treats the filtrate with an equal measure of hydrochloric acid A dilute solution of bleaching powder is then cautiously added, until the blue colour no longer increases On

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[1] Indoxyl sulphuric acid is described by some writers as indoxyl sulphonic acid The latter name would be applicable to a body of the constitution $C_8H_4(SO_3H)(NH) OH$ This would be isomeric with indoxyl sulphuric acid, and would not exhibit the readiness of the latter in hydrolysing into sulphuric acid and indoxyl (page 6)

[2] Decomposing urine occasionally forms a bluish red pellicle, and ultimately deposits microscopic crystals of indigo blue A calculus of the same nature has been described

agitating with chloroform the colouring matter is taken up and can be obtained on evaporation   Jaffe's method is not suitable for the detection of traces of indigogen, as the colouring matter is destroyed by the least excess of the oxidising agent   Hence MacMunn boils the urine with an equal measure of hydrochloric acid and a few drops of nitric acid, cools, and agitates with chloroform   The chloroform is generally coloured violet, and, when examined in the spectroscope, shows two broad absorption-bands, one on either side of the D line   The less refrangible is due to indigo-blue and the more refrangible to indigo-red, though it is doubtful if the latter colouring matter is identical with the indirubin which occurs in commercial indigo [1]

A C Méhu (*Jour Pharm* , [5], vii 122) adds to the urine about 0 5 c c of strong sulphuric acid to 1 litre of the sample, and then saturates the liquid with powdered ammonium sulphate, whereby any indigotin or indirubin is precipitated [2]   On treating the precipitate in the cold with proof-spirit the indirubin will be dissolved, while the insoluble indigotin is purified by washing with water, followed by spontaneous drying   Méhu proposes a colorimetric process for the estimation of indigotin, which he dissolves in

[1] For the detection of indirubin, O Rosenbach (*Jour Chem Soc* , lviii 1032) adds nitric acid to the boiling urine, cools, adds a large excess of ammonia, and agitates with ether, which will acquire a purple colour if indirubin be present   For its isolation, Rosenbach treats the fresh urine with lead acetate, heats the filtered liquid to boiling, and adds nitric acid, drop by drop, until a purple colour is produced, carefully avoiding excess of acid   The liquid is then cooled and treated with ammonia till alkaline   The precipitate is filtered off, washed in succession with ammonia, dilute hydrochloric acid, and water, and then dissolved in boiling alcohol   The solution deposits indigo blue on cooling   It is filtered and the filtrate treated with alcoholic lead acetate, again filtered, and most of the alcohol boiled off   On diluting the residual liquid with water, impure indirubin is precipitated as a brown powder, which, after washing with water, may be purified by crystallisation from chloroform or ether

[2] Apparently, Méhu's method is intended to apply to ready formed indigotin and indirubin, but in Michailoff's process it appears to be the indigogens which are precipitated by ammonium sulphate

hot carbolic acid, to which sufficient glycerin or absolute alcohol has been added to prevent crystallisation on cooling   The colour of a solution of indigo-blue of known strength, prepared in this manner, is compared with that of the urinary pigment

W Michailoff (*Jour Chem Soc*, liv 880) also saturates the acidified urine with finely powdered ammonium sulphate, and then extracts the urobilin by repeated agitations with ethyl acetate (acetic ether) The aqueous layer is next mixed with an equal measure of fuming hydrochloric acid, chloroform added, and then cautiously treated with dilute bromine-water, agitating well between each addition   By presenting the indigo with the solvent when in the nascent state its extraction is said to be very readily and perfectly effected [1]

Indoxyl-sulphuric acid occurs in very small quantities in normal human urine, Jaffe finding from 004 to 0 019 gramme in 1500 c c of the excretion Horse's urine contains twenty-three times as much [2] The proportion in human urine is much increased in certain diseases, such as cholera, typhus, peritonitis, dysentery, and Addison's disease   In obstructive diseases of the small intestine the increase is enormous   The presence of a large amount of indigogens in the urine generally implies that abundant albuminous putrefaction is in progress in some part of the system, these putrefactive products being absorbed and eliminated by the kidneys in the forms of i n d o x y l - s u l p h u r i c   a c i d   and   its   analogue

[1] All the oxidising agents mentioned in the test are liable to destroy the indigo blue if used in excess   A preferable plan is to employ ferric chloride in presence of hydrochloric acid

From 25 litres of normal dogs' urine, J Hoppe Seyler (*Jour Chem Soc*, xlvi 1058) isolated 1 gramme of crystallised potassium indoxyl-sulphate and 0 5 gramme of potassium phenyl sulphate   Neither orthocinnamic acid, orthoamidocinnamic acid, or orthonitrobenzaldehyde, alone or with acetone, produced any increase in the quantity of indigogens excreted

s k a t o x y l - s u l p h u r i c   a c i d, $C_8H_6C(H_3)N\,SO_4H$
The latter body is also found in sweat, and is said
to be somewhat more abundant in human urine than
the indoxyl-compound   When decomposed by hydro-
chloric acid or an oxidising agent, it gives a colour-
ing matter usually reddish, but which may possess a
marked purple tint

Traces of compounds of indoxyl and skatoxyl with
g l y c u r o n i c   a c i d (page 37) not improbably exist
in normal urine, and their proportions appear to be
greatly increased under certain conditions

APPENDIX

Weights and Measures.

1 grain, gr
1 ounce, oz = 437 5 grains
1 pound, lb = 16 ounces = 7000 grains

ENGLISH MEASURES OF CAPACITY

1 minim   (min)   = 0 91146 grain-measure
1 fluid drachm (fl drm) = 60 minims
1 fluid ounce (fl oz) = 8 fluid drachms
1 pint   = 20 fluid ounces
1 gallon   = 8 pints

The term *fluid grain* is not official, but is sometimes used to denote the volume occupied by 1 grain weight of distilled water

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METRIC SYSTEM OF WEIGHTS AND MEASURES

The basis of the metric system is the length of one ten-millionth of a quadrant of a meridian of the earth's surface, passing through Paris   This length is called a *metre,* and is equal to 39 37079 inches, or very nearly the length of a pendulum vibrating seconds

The sub divisions of the metre are the *decimetre* (= 3 937 inches), the *centimetre* (0 3937 inch), and the *millimetre* (= 0 03937 inch)

A *gramme* is the weight of water which occupies, at 4° C , a cube measuring 1 centimetre in the side

1 milligramme = $\frac{1}{1000}$ part of a gramme, or 0 001 grm

1 kilogramme = 1000 grammes = the weight of 1000 c c or 1 *litre* of water at 4° C

Relation of English Weights to Metric Weights

1 grain	=	0 0648 gramme	or	64 8 milligrammes		
2 ,,	=	0 1296 ,,	,,	129 58	,,	
3 ,,	=	0 1944 ,,	,,	194 37	,,	
4 ,,	=	0 2592 ,,	,,	259 16	,,	
5 ,,	=	0 3239 ,,	,,	323 95	,,	
6 ,,	=	0 3887 ,,	,,	388 74	,,	
7 ,,	=	0 4535 ,,	,,	453 53	,,	
8 ,,	=	0 5183 ,,	,,	518 32	,,	
9 ,,	=	0 5831 ,,	,,	583 11	,,	
10 ,,	=	0 648 ,,	,,	648 0	,,	

1 ounce = 437 5 grains = 28 3495 grammes
1 pound = 7000 grains = 453 5927   ,,

Relation of Metric Weights to English Weights

1 milligramme	=	0 01543 grain
1 gramme	=	15 4323 ,,
2 ,,	=	30 8647 ,,
3 ,,	=	46 2970 ,,
4 ,,	=	61 7294 ,,
5 ,,	=	77 1617 ,,
6 ,,	=	92 5941 ,,
7 ,,	=	108 0264 ,,
8 ,,	=	123 4588 ,,
9 ,,	=	138 8911 ,,
10 ,,	=	154 3234 ,,

1 kilogramme = 15432 349 grains = 2 lbs 3 oz 119 8 grains

Relation of English Measures to Metric Measures

1 minim	=	0 05916 cubic centimetre		
1 fluid drachm	=	3 5495	,,	,,
1 fluid ounce	=	28 396	,	,,
1 pint	=	567 92	,,	,,
1 gallon	=	4543 36	,,	,, , or 4 5434 litres

RELATION OF METRIC MEASURES TO ENGLISH MEASURES

1 cubic centimetre = 16 9034 minims, 15 432 grain-measures, or
0 28172 fluid drachm

1 litre = 1000 c c = 281 72 fluid drachms or 35 2154 fluid ounces

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RELATION OF ENGLISH MEASURES TO ENGLISH WEIGHTS

1 minim is the measure of 0 91146 grain of water at 60° F

1 grain-measure ("fluid grain") is the volume occupied by 1 grain
of water at 60° F

1 fluid drachm is the measure of 54 6875 grains of water

1 fluid ounce	,,	,,	437 5	,, or 1 ounce of water
1 pint	,,	,,	8750 0	,, or 1 25 pound of water
1 gallon	,,	,,	70000 0	,, or 10 pounds ,,

CONVERSIONS

To convert *grammes per* 100 *c c* of liquid into *grains per gallon*,
multiply by 700

To convert *grammes per* 100 *c c* of liquid to *grains per fluid
ounce*, multiply by 4 375

To convert *grammes per litre* of liquid into *grains per gallon*,
multiply by 70

To convert *grains per gallon* of liquid into *grammes per litre*,
divide by 70

Relations of Thermometric Degrees.

To convert degrees Fahrenheit into degrees Centi-
grade, subtract 32, multiply the remainder by 5, and divide the
product by 9, or $C = (F - 32) \times 5 - 9$

To convert degrees Centigrade into degrees Fahren-
heit, multiply by 9, divide by 5 and add 32, or $F = \frac{9}{5} C + 32$

Tensions of Aqueous Vapour in Millimetres of Mercury.

°C	mm	°C	mm	°C	mm	°C	mm
10 =	9 126	14 =	11 882	18 =	15 351	22 =	19 675
11 =	9 751	15 =	12 677	19 =	16 345	23 =	20 909
12 =	10 421	16 =	13 519	20 =	17 396	24 =	22 211
13 =	11 130	17 =	14 409	21 =	18 509	25 =	23 582

Symbols and Combining Weights of Elements.

Element	Symbol	Combining Weight	Element	Symbol	Combining Weight
Barium,	Ba	137	Magnesium,	Mg	12
Bromine,	Br	80	Manganese,	Mn	55
Calcium,	Ca	40	Mercury,	Hg	200
Carbon,	C	12	Nitrogen,	N	14
Chlorine,	Cl	35 5	Oxygen,	O	16
Copper,	Cu	63	Phosphorus,	P	31
Gold,	Au	196 5	Platinum,	Pt	194
Hydrogen,	H	1	Potassium,	K	39 1
Iodine,	I	127	Silver,	Ag	108
Iron,	Fe	56	Sodium,	Na	23
Lead,	Pb	207	Sulphur,	S	32
Lithium,	Li	7	Zinc,	Zn	65

The combining weights in the above table are in most cases the nearest whole numbers, compared with oxygen as 16   The figures are sufficiently exact for the purposes of this work, but make no pretence to rigidly accurate expression

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Normal and Standard Solutions.

A n o r m a l s o l u t i o n is one containing in 1000 cubic centimetres (= 1 litre) such an amount of its active constituent as will combine with, replace, or oxidise 1 gramme of hydrogen Hence normal (expressed $\frac{N}{1}$) solutions of the following substances have the strengths given on next page

A n o r m a l s o l u t i o n may also be described as one containing the hydrogen-equivalent in grammes of the essential substance, with the addition of sufficient distilled water to make up the final volume at 60° F (= 15 5° C) to 1 litre (= 1000 c c)

D e c i n o r m a l s o l u t i o n s are $\frac{1}{10}$ of the strength of normal solutions, and are expressed thus, $\frac{N}{10}$

C e n t i n o r m a l s o l u t i o n s are $\frac{1}{100}$ of the strength of normal solutions, and are expressed thus, $\frac{N}{100}$

			GRAMMES PER LITRE
Normal caustic soda	contains Na		= 23 0
,, ,, ,,	,,	NaOH	= 40 0
,, ,, potash	,,	KOH	= 56 1
,, sodium carbonate	,,	$\dfrac{Na_2CO_3}{2}$	= 53 0
,, hydrochloric acid	,,	HCl	= 36 5
,, sulphuric acid	,,	$\dfrac{H_2SO_4}{2}$	= 49 0
,, oxalic acid	,,	$\dfrac{H_2C_2O_4,2H_2O}{2}$	= 63 0
Decinormal silver nitrate	,,	$\dfrac{AgNO_3}{10}$	= 17 0
,, potassium permanganate	,,	$\dfrac{KMnO_4}{5 \times 10}$	= 3 162

Liquor Potassæ and *Liquor Sodæ*, B P , are approximately normal solutions, the former containing 61 8 grammes of KHO and the latter 43 grammes of NaHO in 1 litre

Other volumetric solutions of definite strength, but not falling within the above classification, are often very convenient for special purposes These *standard solutions* are usually designed to measure one particular substance, and are generally prepared so that each cubic centimetre will react with 0 001 or 0 010 gramme of the substance to be measured Thus, if 4 789 grammes of silver nitrate be dissolved in distilled water, and the solution diluted with distilled water to exactly 1 litre (= 1000 c c) at 60° F , each 1 c c of the solution will exactly precipitate 0 001 gramme of chlorine

These standard solutions are simply reciprocals of normal solutions , that is, they have the strength of normal solutions divided by the hydrogen-equivalent of the substance to be measured Thus, 1 c c of *normal* silver nitrate solution precipitates 35 5 milligrammes of chlorine , but if diluted 35 5 times it will give a *standard* solution 1 c c of which will precipitate 1 milligramme of chlorine Such solutions are of great utility in the practical work of the laboratory

A good example of the application of such a standard solution is afforded by the method employed for determining the proportion of *chlorides in urine* Direct precipitation of the sample by silver nitrate is not applicable, since much organic matter is thrown down together with the silver chloride The simplest process generally

applicable is to evaporate 20 c c of the urine to dryness in platinum with 3 or 4 grammes of nitre (potassium nitrate) free from chlorides   On gently heating the residue, the organic matter is oxidised by the oxygen of the nitre, and on raising the temperature to incipient redness complete combustion of the carbonaceous matter results, and a perfectly white product is obtained   This, when cold, is treated with hot water, the solution acidulated with nitric acid, a little prepared chalk added, and the whole thoroughly agitated till neutral to litmus   The liquid is then diluted to 100 c c, and passed through a dry filter   Fifty c c of the filtrate ($=10$ c c of the original urine) should then be placed in a porcelain basin and two drops of a saturated solution of neutral potassium chromate added   A standard solution of silver nitrate containing 4 789 grammes of pure $AgNO_3$ per litre is then gradually added, with constant stirring, until the lemon-yellow colour of the contents of the basin changes to reddish-yellow   This point indicates the conversion of the whole of the chlorides present into white silver chloride, AgCl, and the commencement of the formation of the red silver chromate, Ag_2CrO_4 Every 1 c c of the silver solution used represents 0 001 gramme of chlorine in the 10 c c of urine employed ($=0$ 01 gramme per 100 c c)   Hence if 37 c c be required, the urine contains 0 37 per cent of chlorine, which figure, multiplied by 4 375, equals 1 62 grains of chlorine per fluid ounce

The chlorine contained in the urine is largely dependent on the quantity of common salt taken with the food, but a portion of it is derived from chlorides of potassium and sodium naturally present in the food   The chlorine found can be calculated into its equivalent of common salt by multiplying it by the factor 1 648 (or, approximately, by dividing it by 0 6)   Bromides and iodides, which are not natural constituents of urine but appear after administration of medicines containing them, react like chlorides with silver nitrate   In cases of pneumonia, the chlorides almost entirely disappear from the urine, while the sputum contains an excessive amount

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ERRATUM

Page 90, line 2, after the word "be" *insert the words* "treated with caustic soda and"

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